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HYBRIDIZATION STUDIES ON A ZINC-INDUCED VARIANT OF HYPO-MYCES IPOMOEAE

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(WITH 2 FIGURES)

The first report of variation in fungi attributable to the presence of a toxic chemical substance in the culture medium was that of Arcichovskij (1). In his investigation, a series of cultures of a normal, black-spored strain of Aspergillus niger was established on Raulin's fluid containing zinc sulfate at a concentration of 0.0001 N which had been found to stimulate growth of this fungus. The spores with which the series was started were obtained from a culture of a single-spore strain of A. niger growing on medium containing 0.025 N zinc sulfate, which was near the maximum concentration for growth. A variant strain which bore vellow-brown, rather than black, spores, and which produced a strong reddishbrown pigmentation of the normally colorless culture fluid appeared in the fifth culture-generation of the series. The variant strain retained its characteristics through 24 transfer generations. The appearance of the variant was believed to have been a result of the initial cultivation of the normal strain on the medium containing a sub-lethal concentration of zinc sulfate. In later experiments on induced variation, Brierley (2) was unable to obtain any permanent alteration in either Aspergillus or Penicillium species, and Tu (7) was unsuccessful in producing variation in Fusarium species, by the addition of zinc sulfate or other toxic substances to

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the substrate. Neither of these workers, however, cultivated the fungi on media containing near-lethal concentrations of zinc salts.

The writer (3) has previously described experiments in which quite stable variants of a Fusarium species appeared as the result of cultivation of the normal strain on media containing sub-lethal concentrations of zinc salts. The nature of this induced variation, whether cytoplasmic or genetic, could not be determined, for the species involved produced no sexual fruits. Hence, hybridization studies with a zinc-induced variant of Hypomyces Ipomoeae have been carried out. This fungus had previously been shown to possess self-sterile strains of opposite sexual reaction which, when mated, readily produced sexual fruits in culture (Dimock, 4). The results of the hybridization investigations are presented in the following pages.

PRODUCTION OF THE ABORTA VARIANT

The experimental procedure employed in the production of the variant may be described briefly. A base medium of the following composition was prepared: sucrose-40 g.; potassium nitrate-8 g.; potassium acid phosphate-5 g.; magnesium sulfate-2.5 g.; agar-agar-60 g.; distilled water to make 1 liter. The molten medium was accurately tubed, 10 c.c. per tube, and sterilized. At the same time, distilled water solutions of zinc nitrate [Zn(NO₂)₂. 6H₂O₁, containing 0.1%, 0.2%, 0.4%, 0.6%, 0.8%, and 0.9% of zinc (Zn), were prepared, accurately tubed in 10 c.c. lots, and sterilized. Petri plates of zinc-containing media were then prepared by simultaneously pouring the contents of one 10 c.c. lot of base medium and one 10 c.c. lot of zinc nitrate solution into each plate and rotating thoroughly to mix the constituents. This technique was necessary because zinc ion is largely precipitated from solution if the zinc salt is added to the base medium prior to sterilization. Since the volume of the solution was doubled by mixing the constituents, the final concentrations of zinc in the different lots became 0.05%, 0.1%, 0.2%, 0.3%, 0.4%, and 0.45%. It should be pointed out that the actual concentrations of zinc ion in the various media were not known. Crystals, presumably of some insoluble zinc salt, were seen to form in the solidified media in a very short time, and to "grow" as the media aged. Concurrently, of course, evaporation of water was taking place, so that the zinc ion concentrations may possibly have remained more or less constant. The actual concentrations of zinc ion were of no concern in the present investigation, but it is reasonably certain that they were from the beginning somewhat below the zinc concentrations given above.

Three isolated microsporelings of each of the two tester-strains of *Hypomyces Ipomoeae*, 3–3 (A) and 3–14 (a) (see Dimock, 4), were planted on each of five plates containing 0.05%, 0.1%, and 0.2% of zinc, and on each of ten plates containing 0.3%, 0.4%, and 0.45% of zinc. Plates of base medium which had been diluted with 10 c.c. of sterile distilled water rather than with zinc solution were similarly planted to serve as checks.

On media containing 0.45%, 0.4%, and 0.3% of zinc, all sporelings save two of 3–3 on a single plate of the last mentioned medium were killed. This was proved by the fact that those which were subsequently transferred to normal malt-extract agar medium failed to grow. Twelve of the fifteen sporelings of 3–3 and four-teen of the fifteen sporelings of 3–14 on medium containing 0.2% of zinc, and all sporelings on media containing 0.1% and 0.05% of zinc, developed colonies.

All colonies save one on medium containing 0.05% of zinc, and all save two on medium containing 0.1% of zinc, were like the *normal* colonies on the check plates except for considerable restriction in growth rate. One 3–14 colony on medium containing 0.05% of zinc was somewhat atypical, and two colonies of 3–3 on medium containing 0.1% of zinc developed sectors. Transfers from these atypical growths to malt-extract agar yielded only *normal* cultures. Colonies on medium containing 0.2% of zinc were greatly restricted in growth, but some eventually developed fast-growing sectors. In spite of repeated transferring from a large number of these sectors, no variant types were obtained.

One colony of 3-14 on medium containing 0.2% of zinc was considerably slower in growth than all the rest, but in other respects was similar to those colonies which failed to develop sectors (Fig. 1). Three transfers to malt-extract agar slopes were made from different points at the margin of this colony. Growth on one of these slopes was of *normal* appearance, while growth on

the other two appeared to be a mixture of *normal* and a variant type of mycelium. The variant component was characterized by more restricted aerial growth and by the production of a bright red diffusible pigment. Repeated sub-culturing was required to obtain a pure culture of the variant. The new strain, designated *aborta*, was proved to be pure and homocaryotic by its constancy through 5 consecutive single-microconidium culture-series consisting of 25 cultures each. All subsequent cultures of this strain have exhibited an unusually high degree of uniformity, having shown no tendency toward "reversion" or further variation.

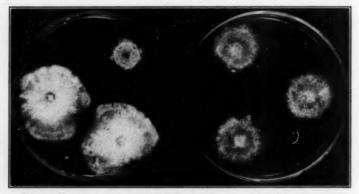


Fig. 1. Petri plates of zinc-containing medium on which the aborta strain was produced. The small colony in the plate on the left yielded a mixed culture containing normal and aborta components. All colonies originally resembled those in the right-hand plate, but many increased in diameter by more rapid subsequent growth. None save the single small colony shown above yielded a variant, however.

The aborta strain differed from normal on malt-extract agar by the development of a light, reddish pigmentation of the aerial hyphae and, after 9 or 10 days, of a bright red pigmentation of the medium. On potato-dextrose agar, the aerial hyphae rapidly exhibited a rather beautiful bluish-purple pigmentation, the cultures became strongly fluted, and a deep purple pigment diffused into the medium (FIG. 2). No perithecial fundaments were observed in cultures of this strain on any of the media employed, and it produced conidia much more sparingly than the normal.

HYBRIDIZATION STUDIES

The aborta strain was mated with both of the normal tester-strains (3-3, A and 3-14, a) in order to determine whether or not it were fertile, whether or not the sexual-reaction factor had been altered, and whether or not the distinguishing morphological characters were heritable. In time perithecia developed in matings of aborta with normal A (3-3) as well as in check matings of the two normal tester-strains. No perithecia were formed in any matings of aborta with normal a (3-14). This proved the sexual reaction of the variant to be the same as that of the normal strain (3-14, a) from which it was derived. The hybrid perithecia were noted to be considerably smaller than normal inbred perithecia, varying from one-third to two-thirds the size of the latter.

Microscopic examination of hybrid perithecia revealed the most striking effect of the aborta strain, namely, that all fertile asci bore 4, rather than 8, ascospores, the latter number being typical of normal inbred asci of H. Ipomoeae. Moreover, very few fertile hybrid perithecia were formed, and in those the number of fertile asci was very low. Three possible explanations of the above phenomenon were suggested: (1) the aborta strain either possessed a factor which inhibited ascospore formation or lacked the factor or factors for ascospore formation; (2) each of the four spores in the hybrid asci contained two of the eight nuclei resulting from the normal division processes accompanying ascospore formation, as is the case in Neurospora tetrasperma and certain other fungi; and (3) the usual third nuclear division preceding delimitation of ascospores was omitted. If the first explanation is correct, no aborta cultures should appear in the progeny of aborta × normal perithecia. If the second explanation is correct, the hybrid population should consist either (a) of pure normal cultures and pure aborta cultures in equal numbers, or (b) wholly of mixed aborta-normal cultures, or (c) of pure normal, pure aborta, and mixed aborta-normal cultures in somewhat uncertain ratios. If the third explanation is correct, the progeny should consist of pure normal and pure aborta cultures in equal numbers.

To clarify this question and provide further information concerning $normal \times aborta$ hybrids, a total of 311 single-ascospores

were isolated from six hybrid perithecia and planted on potatodextrose agar slopes. Each spore was examined under the microscope, in most cases using the 45 × objective, to insure its purity before transferring to the agar slope. Since the hybrid perithecia were ostiolate and discharged their spores spontaneously, it seemed reasonable that these spores, like conidia (Dimock, 4), might function in the spermatization of the perithecial fundaments produced by the normal parent hyphae. If such secondary perithecia were formed, many of them would be inbred rather than hybrid. All perithecia to be used for ascospore isolation were therefore crushed in sterile water and examined microscopically prior to spore isolation as a precaution against unwittingly employing normal inbred perithecia in place of aborta × normal hybrids. This precaution was fully justified by subsequent observations which showed that after the first hybrid perithecia were produced, secondary normal inbred perithecia developed in such abundance that it became difficult to find hybrids.

Of the 311 single ascospores thus obtained from normal X aborta perithecia, 273 developed normal cultures, 32 developed "purple" type cultures, 4 developed distinctly new types of cultures, and 2 gave rise to cultures which appeared to be of the aborta type. The "purple" type cultures were similar to those which had been obtained from normal inbred perithecia in earlier studies (Dimock, 4). The four new types were quite distinct from one another and from the two parent strains, and did not appear to be intermediates. The "purple" type and the four new types will be discussed more fully in a later paper. In anticipation, however, it should be noted here that the appearance of the "purple" type apparently results from the conversion of a "mutable gene" of the normal complex during maturation of the zygote nucleus, and that such conversion is reversible. Hence, in a discussion of normal vs. aborta, the "purple" type may be considered as normal.

The high percentage of normal cultures (including "purple" type) in the f_1 population, namely, 98 per cent, suggested that the theoretical expectancy should be 100 per cent, or in other words, that the first explanation offered to account for the four-spored character of aborta \times normal asci was correct. Were such the

case, no aborta cultures should appear in the hybrid progeny; hence, the two aborta cultures which did appear received special attention. A very important feature of these cultures was that each had a sector of apparently normal growth originating from the inoculum. Single-spore isolations proved that in each case both aborta and normal hyphae were present. This observation indicated that in these cases a germinating aborta microspore had been carried over with a normal ascospore. Were this true, doubt would be cast upon the reliability of the entire investigation. The alarm thus caused was dispelled when it was determined that one of the aborta cultures bore sexual-reaction factor A, and the other the factor a. Conidial contaminants could have borne only the factor a. The normal components of these cultures possessed the same sexual-reaction factors as the respective aborta components.

The above observations led to the conclusion that the two ascospores yielding mixed aborta-normal cultures contained both normal and aborta nuclei. Such a condition could arise if, on rare occasions, more than one nucleus were included in a single ascospore as the result of some abnormality in ascospore delimitation in hybrid asci. If, then, an aborta nucleus were fortuitously included in an ascospore delimited by a normal nucleus, the developing mycelium would be mixed, containing both aborta and normal components, as in the above cases. The fact that aborta strains appeared in the f, population only in such mixed cultures offers strong support to the hypothesis, previously stated, that aborta nuclei either lack the factor for ascospore delimitation or possess its recessive allelomorph. The hypothesis is further supported by the fact that in crosses between the two f, aborta strains and the normal tester-strains the primary perithecia bore only 4-spored asci, just as in the original hybridization.

If the assumption is valid that ascospores including more than one nucleus may in rare instances be delimited in $normal \times aborta$ asci, it might well be proposed that nuclei bearing opposite sexual-reaction factors would occasionally be included in a single ascospore. This indeed proved to be the case, for in two single-ascospore cultures of the above population fertile perithecia were formed. The possibility that contamination had occurred in these two cases cannot, unfortunately, be disproved. It may be recalled,

however, that each bit of agar bearing an isolated germinating ascospore was given careful microscopic examination prior to transfer to the culture tube. Furthermore, although over 1000 single-ascospore cultures from both inbred and hybrid perithecia have been critically observed during this investigation, perithecia



Fig. 2. Three single-conidium cultures each of normal and aborta strains on potato-dextrose agar. Upper, cultures 4 days old; lower, cultures 9 days old.

were found only in these two single-ascospore cultures derived from $aborta \times normal$ hybrid perithecia.

The distribution of the sexual-reaction factors in the other 309 f_1 cultures is indicated in Table 1. Since it has been shown

(Dimock, 4) that sexual reaction in *Hypomyces Ipomoeae* is determined by a single allelomorphic factor-pair, a one to one distribution of sexual-reaction factors should be expected in the present case unless the genes for ascospore delimitation and those for sexual reaction are linked. The data indicate that no such linkage exists, for were such the case a cross-over value far in excess of 50 per cent would have to be assumed to explain the observed A/a ratio.

 ${\bf TABLE~1}$ Factor Distribution in the Progeny of normal \times aborta Perithecia

Perithecium	Normal ^a		Aborta b	
Peritnecium	LA c	La	1A	la
Т3	25	37	0	0
T4	3	5	0	0
T5	19	33	0	0
T6	53	64	0	1
T7	20	23	0	0
Т8	15	10	1	0
Totals	135	172	1	1

a Includes also new variant types other than aborta.

^b Actually "mixed" ascospores. *Normal* components not considered separately.

^c I = recessive for *aborta* characters, L = dominant *normal* allelomorph; A/a = sexual-reaction factors.

The f_1 aborta strain which bore sexual-reaction factor A was backcrossed with the aborta parent strain which, as noted, bore sexual-reaction factor a. No perithecia, nor even perithecial fundaments, were produced in such matings. The difference in sexual reaction between the two aborta strains was verified by repeated matings with the normal tester-strains.

DISCUSSION

The foregoing work has shown that variation of a striking sort may be induced by cultivating gameophytic mycelium of $Hypo-myces\ Ipomoeae$ on medium containing a sub-lethal concentration of a zinc salt. The claim is not that the mutation considered was a specific effect of zinc ion, the writer inclining to agree with Schiemann (6) and Waterman (8) that the effect is one of dis-

turbed metabolic processes which might result from any one of many factors.

The outstanding feature of the mutant strain, aborta, is that 4, rather than 8, spores are delimited in each fertile normal × aborta ascus. The data suggest that this phenomenon may best be explained by the assumption that a single gene or gene-complex determining ascospore delimitation has either been deleted or inactivated. Dodge (5) proposes that a somewhat similar strain of Neurospora tetrasperma, whose parentage may be traced to an abnormal strain which originated from an X-ray-treated ascospore, bore a recessive factor, l, lethal for spore formation in asci homozygous for this factor. Normal strains of N. tetrasperma were assumed to bear its dominant allelomorph, L, which determined ascospore delimitation. Thus, in matings between l strains bearing opposite sexual-reaction factors, ascospore abortion was complete, whereas in hybrid $(Al \times aL \text{ or } AL \times al)$ asci numerous ascospores were formed. It is significant that in such hybrid asci some uninucleate spores were delimited whose single nuclei bore only the allelomorph l; hence, the effect of this factor was not a simple inhibition of ascospore delimitation, but rather, as Dodge demonstrated cytologically, an interference with maturation of the zygote nucleus such that the eight daughter nuclei in asci homozygous for l disintegrated and thus could not delimit spores.

The aborta strain of Hypomyces Ipomoeae differs from the N. tetrasperma strain described by Dodge in two respects: (1) neither perithecia nor asci are formed in matings between aborta strains of opposite sexual reaction; (2) aborta nuclei are incapable of delimiting spores in normal \times aborta hybrid asci, but may in rare instances be fortuitously included in ascospores delimited by normal nuclei. The present observations, however, warrant the assumption that the normal strain bears a factor or factor-complex determining ascospore delimitation which, following Dodge, will be designated L, and that the aborta strain bears its recessive allelomorph, l, which effects inhibition of ascospore delimitation. The striking morphologic characters exhibited by aborta are apparently determined by the same factor, l, or by a closely linked factor. While the appearance of the aborta cultures in the l_1 progeny of the normal \times aborta hybrids at first suggested crossing-

over between factors for ascospore delimitation and factors for morphologic character, the suggestion was proved groundless by the fact that only 4-spored asci were formed in the hybrid asci which developed when these two cultures were mated with the *normal* tester-strains.

It should be noted that the aborta strain is not fitted to compete successfully with the normal strain under the environmental conditions prevailing in this investigation. This is evident from the facts (1) that conidium formation by the aborta strain is quite poor when compared with the normal, (2) that the aborta strain does not inbreed, and (3) that aborta nuclei in hybrid asci do not delimit spores. A chance observation bearing on this point should be of interest. The original f, aborta A culture was, as previously noted, predominantly of the aborta type, but possessed a small wedge sector of normal mycelium in the upper half of the tube. Twenty single conidia were isolated from the upper portion of this culture when it was 6 days old. Of these, 18 developed aborta and 2 developed normal cultures. All of these aborta A cultures were by accident discarded, and to recover the strain in pure culture, 20 more single conidia were isolated from the lower portion of the original culture when it was 25 days old. This time 19 of the developing cultures were normal, and only one was aborta. Close scrutiny of the original culture revealed that the normal mycelium had grown down over the aborta mycelium to within about 1 cm. of the bottom of the latter. Twelve single microspores were therefore isolated from this remaining centimeter of aborta growth when it was 29 days old. All of these yielded pure aborta cultures. In time the normal completely overgrew the aborta mycelium in the original culture.

SUMMARY

A striking variant has been produced by cultivation of monoploid hyphae of *Hypomyces Ipomoeae* on medium containing a sub-lethal concentration of zinc nitrate.

Analyses of 5 consecutive single-conidium culture-series, consisting of 25 cultures each, have proved the mutant strain, aborta,

to be homocaryotic. Single-conidium and mass-transfer cultures of the *aborta* strain have shown a high degree of uniformity.

The most striking feature associated with the variant was that four, rather than eight, spores were formed in $aborta \times normal$ asci, due apparently to inability of nuclei carrying the aborta factors to delimit spores.

Evidence strongly indicated that in rare instances ascospores in $aborta \times normal$ asci may include more than one of the eight nuclei resulting from maturation of the zygote nucleus. Thus, in two instances, single ascospores isolated from hybrid perithecia yielded mixed cultures containing both normal and aborta hyphae. The two f_1 aborta strains thus obtained bore opposite sexual-reaction factors. In two other instances, single ascospores from hybrid perithecia yielded cultures containing normal hyphae of both sexual reactions, as was evidenced by the appearance of fertile perithecia in both cultures.

The inability of *aborta* nuclei to delimit ascospores may be attributed to the inactivation or to the deletion of one or more genes determining ascospore delimitation. The ascospore-delimitation factors and the sexual-reaction factors are in different linkage groups.

The genic alteration or mutation, although having occurred on medium containing a sub-lethal concentration of zinc, cannot be considered a specific effect of zinc ion without further evidence.

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TWO CANADIAN COLLECTIONS OF CANTHARELLUS MULTIPLEX 1

IRENE MOUNCE AND HENRY A. C. JACKSON
(WITH 1 FIGURE)

In 1898 Mrs. Elizabeth W. Woodworth collected an unusual fungus in a Maine woods and made the following notes about it: "Growing in a large irregular mass and weighing one to three pounds. . . . The color of the fresh pileus was dull purple or purplish lead color, the flesh was decidedly purple, tender and brittle; spores white or whitish, very abundant, dusting the entire plant; height six to twelve inches; taste mild, odor aromatic. The plant suggested to me curly cabbage . . . every curly edge having a silver line, perhaps from the light colored spores. . . ."

In 1899 Underwood (1) assigned the name Cantharellus multiplex to this fungus and published the following description:

"Cespitose-multiplex from a compact base which is nearly black when dry; pilei more or less flabellate, compound, 3–5 cm. wide, nearly as long, blackish above in drying, cinereous beneath and concolorous to the base of the stipe where it joins the blackish base; stipe 2–4 cm. long, often deeply grooved above by the decurrent margins of the pileus, occasionally somewhat tubular by their union along the outer edges; hymenium radiately venulose-reticulate with irregular cross veinlets and frequent minute slit-like fissures and larger irregular depressions; spores copious, 5–6 μ in diameter often appearing coarsely lobed when freshly moistened as though formed of united granules.

"On the ground in dense woods of spruce and fir, Seal Harbor, Mt. Desert, Maine, August 1898."

He noted, however, "the plant is a remarkable one and from its habit might well form a distinct genus since it has little in common with *Cantharellus* except its fold-like gills."

In 1910 Murrill (2) founded the genus *Polyozellus* consisting of the single species *P. multiplex* on these specimens collected by

¹ Contribution No. 483 from the Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa, Canada.

Mrs. Woodworth. He failed, however, to mention the characteristic rough walled spores.

Since, as far as we are aware, no further collections of this



Fig. 1. Cantharellus multiplex Underwood [Polyozellus multiplex (Underw.) Murr.]. A drawing of the St. Come specimen made from fresh material. Natural size.

fungus have been recorded, two which were made in Quebec recently may be of interest. The first of these collections was made by the junior author who found the specimen, illustrated in the accompanying drawing (FIG. 1), beneath trees in a mixed woods at St. Come, Quebec, on September 8, 1935. The specimen is now in the herbarium of the Division of Botany. The second collection was sent to the Division of Botany by Mrs. Charles A. Lewis, who found the fungus growing in "a mass of one to three feet" in a dry spruce woods at Metis Beach, Quebec, on July 31st, 1936. Parts of this collection have been sent to The New York Botanical Garden, the Farlow Herbarium, Pennsylvania State College Herbarium, and to The Herbarium at Kew. None of the specimens reaches the height as given by Mrs. Woodworth but the form, color, and spores are characteristic of this species.

Our thanks are due to Dr. L. O. Overholts for identifying the Metis specimen and comparing it with type material kindly loaned by Dr. F. J. Seaver, and to Mrs. Charles A. Lewis who, at a good deal of inconvenience to herself, obtained further material for us from Metis Beach.

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HYDROGEN ION CONCENTRATION AND ASCUS FORMATION

LEWIS B. LOCKWOOD 1

In the course of a study of the nutrition of *Penicillium (Carpenteles) javanicum* van Beijma, the cultures were maintained on cornmeal agar. Perithecia were produced abundantly on this medium. The fungus also produced an indicator pigment, the structure of which is not known. When the agar medium is made with the clear extract of cornmeal, the pH of the medium is such that the pigment is red, but if some debris or solid part of the cornmeal is allowed to mix with the agar, the color is yellow. It was observed that if some cornmeal debris were allowed to settle into the bottom of a test tube before pouring the plates, the debris was not spread uniformly throughout the plate, thus giving areas in which each color prevailed. Examination of perithecia from such a plate revealed that most of the perithecia from the red areas were filled with asci.

To further investigate this phenomenon, Penicillium javanicum, Aspergillus herbariorum ser minor (Mangin) Thom and Church, and Chaetomium globosum Kunze were grown on synthetic media with the pH regulated by a modified McIlvaine's system of buffers as follows:

pH ² 0.1 M Citric Acid	0.2 M K ₂ HPO ₄			
cc.	cc.			
2.1	0			
3.145	15			
4.035	25			
5.0	32			
6.1	40			
7.1 8	52			
8.5 0	60			

¹ 270th Contribution from the Industrial Farm Products Research Division, Bureau of Chemistry and Soils, United States Department of Agriculture, Washington, D. C.

³ Determinations were made with a glass electrode by Dr. James McLaren of this Division.

Nutrients were added, and the volumes of solutions made up to 75 cc. For *P. javanicum* and *A. herbariorum* ser. minor, 10 cc. buffered nutrient medium was transferred to each Petri dish which containued 10 cc. washed silica gel. For *C. globosum*, 10 cc. buffered nutrient solution was added to three sterile 9 cm. filter papers in each Petri dish. The nutrient medium for *P. javanicum* contained in addition to the buffers, 250 gms. glucose, 4 mgs. H₃PO₄, 40 mgs. MgSO₄.7H₂O, 8 mgs. KCl and 18 mgs. NH₄NO₃ per liter. For *A. herbariorum* ser. *minor* and *C. globosum*, the nutrients were 50 gms. glucose, 80 mgs. MgSO₄.7H₂O, 8 mgs. H₃PO₄, 16 mgs. KCl and 18 mgs. NH₄NO₃ per liter. Cultures of *P. javanicum* were 21 days, of *A. herbariorum* ser. *minor* 25 days, and of *C. globosum* 26 days old at harvest. All cultures were made in triplicate.

Counts of all mature perithecia in several areas of each plate were made. Data presented in Table I show that in the three organisms, the greatest percentage of ascus-bearing perithecia occurs toward the alkaline range. The color of the indicator pigment of *P. javanicum* is yellow in acid media, changing to red in alkaline media at about pH 6.

No variation in the number of perithecia produced which might be attributed to variation in hydrogen ion concentration was observed.

TABLE I
FERTILITY OF PERITHECIA OF THREE FUNGI GROWN AT VARIOUS HYDROGEN
ION CONCENTRATIONS

	Penicillium javanicum	A. herbariorum	C. globosun		
pH	Color 1	Per Cent with Asci	ser. minor Per Cent with Asci	Per Cent with Asci	
2.1	Baryta Yellow (Pl. IV)	1.0	2	2	
3.1	16 11 11	0.5	_2	2	
4.0	44 46 46	1.0	0	2	
5.0	11 11 11	9.0	59	2	
6.1	Pale ochraceous buff (Pl. XV)	4.0	84	1	
7.1	Light jasper red (Pl. XIII)	73.2	100	16	
8.5	—3	97.7	100	44	

¹ Ridgeway, Robert, 1912, Color Standards and Color Nomenclature. Washington, D. C.

2 No growth.

³ The brown color of the caramelized sugar prevented matching this color.

PASCHER AND THE GENUS ASTERO-CYSTIS OF DE WILDEMAN

J. S. KARLING

In 1893 De Wildeman described Asterocystis as a new genus of chytrids for an Olpidium-like species with somewhat starshaped resting spores. This genus was recognized by most students of the Olpidiaceae for two decades, but in 1917 Pascher, the well known European algologist, published a short article maintaining that the same name had already been given by Gobi (1879) to a red alga of the family Bangiaceae with stellate chromatophores. He accordingly suggested that Asterocystis in the sense of De Wildeman be dropped, and proposed as an alternative the more descriptive name Olpidiaster. His suggestion was adopted by Hösterman and Noack (1923), Kirchner (1923), Heald (1926, 1933), Gäumann (1926), Gäumann and Dodge (1928), Fitzpatrick (1930), Hildebrand (1934), and others. Fitzpatrick in particular calls attention in his synonymy to the claim that Asterocystis De Wildeman is antedated by Asterocytis Gobi.

A careful comparison, however, of the two names shows at once that Gobi's genus differs by the lack of an "s" in the third syllable, and it is thus obvious that on the basis of orthography there is no ground for dropping De Wildeman's generic name. Pascher's contention is difficult to understand in view of the fact that in 1895 (p. 226) De Wildeman called specific attention to this difference; and Pascher himself was apparently aware of it (1925, Heft 11: 159) in his book on the freshwater algae of Germany, Austria and Switzerland. The orthography of the two names is none the less almost the same, and Pascher at first glance apparently came to the conclusion that they were identical. As far as I have been able to determine he has never corrected this error of 1917. Obviously, those who subsequently adopted Olpidiaster as an alternative accepted Pascher's statement as correct, and did not examine the original descriptions of the two genera.

While the generic name Olpidiaster is perhaps more descriptive of the complete life cycles of the species to which it relates than Asterocystis, and its adoption would doubtless eliminate the possibilities of confusion with Gobi's genus, there seems to be no good reason in light of present day knowledge for recognizing either of them as distinct from Olpidium and as a well-defined, valid chytrid genus. De Wildeman's original description related only to the resting spores of Asterocystis, which he claimed differed from those of Olpidium by being stellate with a thin membrane or envelope, possessing a large refractive globule in the center, and not becoming plasmolysed by treatment with glycerine. The first of these characters, however, does not appear to be specific or generic, since Woronin (1878), Dangeard (1886), Nemec (1912), Bensaude (1923) and Bartlett (1928) have found stellate spores in Olpidium also. Thus, as Guyot (1927) has already pointed out the distinction on the character of the resting spore no longer seems tenable. Guyot has furthermore shown in his extensive study that the refractive globule varies in size in Asterocystis, and that the resting spores can be readily plasmolysed; thus breaking down De Wildeman's other two generic distinctions.

In 1901 Marchal discovered the zoösporangia of Asterocystis for the first time, and described them as lacking exit tubes for the liberation of the zoöspores. The latter, according to him, escaped through a lateral opening near one of the extremities, and on the basis of this new character he maintained that the two genera could be more clearly separated. It is to be noted, however, that since his time species of Olpidium have also been described (Kusano, 1912; Schwartz and Cook, 1928) without exit tubes or with only short papillae for the liberation of the zoöspores. Furthermore, Guyot, Vanterpool (1930) and others have since shown that long exit tubes may frequently develop in Asterocystis also. These recent studies have also shown that there is no fundamental difference in the size, shape and appearance of the zoösporangia and zoöspores.

These similarities together with the fact that species of both genera have been reported to parasitize the same hosts and produce similar effects, indicate, it seems to me, that there is no basic distinction between the two as far as our knowledge goes at the present time. Perhaps as Guyot has suggested, future cytological studies may justify de Wildeman's genus, but there is no good evidence at hand to separate it from *Olpidium* at the present time. The number of root inhabiting species of *Olpidium* has been rapidly increasing in the past few years, and it is becoming imperative that intensive cross inoculation experiments coupled with morphological and cytological studies be made before we can determine the validity of the new species conclusively.

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OBSERVATIONS ON A MONASCUS ISOLATED FROM RUBBER ¹

ARTHUR L. SCHADE

Late in the summer of 1931, a specimen of crude rubber of Hevea brasiliensis at the Harvard Botanical Museum was observed to be covered by a heavy white growth. Among the fungi responsible for this growth was one which, on examination, was found to be Monascus ruber van Tieghem. In addition to the moulds, mites were abundant on the specimen. Since the mould flourished wherever the mites had eaten their way into the rubber, it was of interest to determine whether the Monascus ruber was growing on a substratum furnished by the mites or on the rubber alone.

This paper reports the growth of *Monascus ruber* on the rubber apart from the mite excrement and gives the results of growth experiments of *Monascus ruber* and *Monascus purpureus* Went on other crude rubbers, on commercial rubbers, and on extracted rubber hydrocarbon.

HISTORICAL

Examination of the literature dealing with the substrata of *Monascus* does not reveal, to the writer's knowledge, any previous report of rubber as a substratum. Van Tieghem (8), who procured his material from a growth on boiled potato, first described the genus in 1884 and named the fungus *Monascus ruber*. Harz (3), in 1890, reported a fungous growth, brought to his attention by a chemist in a candle and soap factory, on a raw glycerin solution of 8–10 per cent concentration. Young (10) includes this fungus, which Harz (3) named *Physomyces heterosporus*, under *Monascus ruber*, a variable species. Another species found on some oil in cans and on skins from a tannery in France was named *Monascus olei* by Piedallu (6). Inspection by Lewis (4) of

¹ Contribution from the Laboratories of Cryptogamic Botany and the Farlow Herbarium, Harvard University, no. 151.

mycelial growth in a bottle of pickles led him to identify the fungus as Monascus Barkeri Dangeard. Yesair (9) found that Monascus purpureus caused the development of red areas on sausages. The Orient, probably for centuries, has made use of both the fermenting properties of Monascus purpureus, together with a yeast, for the manufacture of alcoholic beverages, and its pigmenting power for the production of colored rice, known in trade as Aga-Koji.

Of some interest is Buchanan's (2) report of Monascus purpureus found in silage in this country. Its occurrence was linked with the death of eleven horses although further investigation did not give evidence of any direct relationship between this particular organism and the death of the horses. Recently, Young (10) has made a study of the physiological characteristics of a Monascus sp. found in maize flour near Johannesburg, South Africa. It is apparent that the genus Monascus, in view of the wide variety of substrata used by the several species, is not closely restricted in its use of nutriment.

MATERIAL AND METHODS

The crude rubber specimen of *Hevea brasiliensis* from which *Monascus ruber* was isolated came from Acre River, Brazil.² On its arrival at the Museum, it was sealed in a glass container. After two years the heavy mould growth was apparent. The presence of *Monascus ruber* in all the mixed cultures of the fungi from the specimen suggests that it was an important constituent of the aggregate growth.

For purposes of comparison, Monascus purpureus, usually associated with a carbohydrate substratum, was isolated from rice grains representative of "Koji" material and employed, along with Monascus ruber, in all of the growth studies. Throughout all of the experiments and subsequent observations on growth, sterile damp chambers were used. Inoculation of the various test materials was most conveniently effected by pipetting onto the

² Through the interest of Professor Oakes Ames, the mouldy crude rubber slab from the Harvard Museum was turned over to Professor Wm. H. Weston, Jr., to whom the writer is indebted for the subsequent outlining of the possibilities for a research problem which the mould growth offered and to whom the writer wishes to express gratitude for his interest and helpful suggestions.

sample one-fourth of a milliliter of inoculum prepared by scraping masses of conidia and ascospores from the surface of stock culture medium of potato-dextrose agar into ten cubic centimeters of sterile water.

EXPERIMENTAL

To determine whether or not Monascus ruber and Monascus purpureus were able to use the crude rubber of Hevea brasiliensis as a substrate, centimeter cubes unaffected by mites and free from fungi, were cut from the slab and inoculated with spore suspensions of the fungi. After three days at 31° C., both species were growing well on the rubber. That the Monascus ruber grew on the crude rubber when separated from the mite material showed that it was not obligately dependent on the activities of the mites for its growth on the museum specimen.

The ability of both species of *Monascus* to grow on a variety of crude rubbers was next investigated. Table I gives the results of this experiment:

TABLE I

GROWTH OF Monascus ruber and Monascus purpureus on Various Crude Rubbers in Damp Chambers at 31° C. after Two Weeks

Type of Rubber M.	ruber	M.	purpureu
Castilloa elastica (unwashed, undried)**		* :	
Castilloa elastica (washed, dried) *			
Urecola elastica***	* *	4 1	
Landolphia sp		*	
Parthenium argentatum		*	* *
Euphorbia lorifolia ***	***	* 1	****
Euphorbia lorifolia		8.1	
Palaquium gutta		*:	

(*)'s indicate relative amounts of mycelial growth.

Despite variations in amounts of growth, all of the crude rubbers inoculated served as nutrient substrata. The differences in growths on the washed and unwashed sample of *Castilloa elastica* may be accounted for by the greater amount of serum ³ present in the unwashed sample.

The ability of samples of commercial rubber, representative of

⁸ That portion of the latex which is not an integral part of the rubber hydrocarbon is called the "serum" and comprises the water, salt, enzyme, protein, sugar, and resin content of the latex. the several stages attained in preparation of the finished vulcanised product from latex, to support growth of *Monascus ruber* and *Monascus purpureus* was next studied. The samples were supplied by the U. S. Rubber Co. They included latex from *Hevea brasiliensis* preserved with ammonia, first latex pale crêpe, smoked sheet, latex-sprayed rubber, and vulcanised samples made from the above three rubbers.

Table II indicates relative amounts of growth on several types of rubber:

TABLE II

GROWTH OF Monascus ON TYPES OF COMMERCIAL RUBBER IN DAMP CHAMBERS
AT 31° C. AFTER TWO WEEKS

		Pale Crêpe	Smoked Sheet	Latex-sprayed	90" vul.	210" vul
4	Mycelium	***	*****	****		_
ube	Conidia	****	*****	*****	-	
M. ruber	Perithecia	* * *	*****	*****		
M	Pigment	*	***	***	_	_
M. pur-	Mycelium	*	* *	* *		
	Mycelium Conidia	*	*		-	-
	Perithecia				-	
0	Pigment	* *	* *	* * *		_

^{(*)&#}x27;s indicate relative amounts.

Monascus ruber seems to develop somewhat better than Monascus purpureus on a rubber substratum especially in the production of perithecia. No growth whatever was produced on the vulcanised samples, a result which is probably due to the presence of sulfur in the rubber since sulfur is recognized generally as inhibitive to growth of fungi. Among the other samples, pale crêpe is least suitable as a nutrient substratum. An explanation of this result may be sought in the method of preparing pale crêpe. The coagulum from the latex is subjected to the action of a stream of water while passing between rollers revolving at uneven speeds so that practically the whole of the serum is washed out. The addition of sodium bisulfite to the latex before coagulation to inhibit the action of an oxidase during the drying of the crêpe may serve the further purpose of inhibiting growth.

The most interesting observation to be made from the above table is the comparative ease with which the fungi made use of the smoked sheet for nutriment. One of the purposes of smoking is to render the rubber less liable to mould attack through the presence of the phenolic constituents in the smoke. Contrarily, the results indicate that the growth of *Monascus ruber* and *Monascus purpureus* is but slightly, if at all, hindered by the smoking of the sheet. The results obtained with the latex-sprayed rubber are what might be expected from a medium possessing all the constituents of the serum in addition to the caoutchouc portion of the rubber. Several unsuccessful attempts were made to grow the fungi on the latex preserved in ammonia. Since, however, growth was produced on latex-sprayed rubber, it is reasonable to infer that the ammonia made the latex unsuitable for support of the fungi and that untreated latex would readily serve as a substrate.

In order to determine whether or not the serum in the rubber was the sole source of nutriment for the growing fungi, it was deemed desirable to separate the caoutchouc from the serum and observe growth on the hydrocarbon alone. The method employed to obtain the rubber hydrocarbon, a polymer of isoprene, involved the use of petroleum ether as the solvent after the resins had been extracted from the crude rubber by acetone. The sol-rubber was then precipitated in 95 per cent ethyl alcohol. Table III gives the results obtained:

TABLE III

OWTH OF Monascus ON SOL-RUBBER HYDROCARBON II

Growth of Monascus on Sol-Rubber Hydrocarbon in Damp Chambers at 31° C. After Two Weeks

	Mycelium	Conidia	Perithecia	Pigment
M. ruber	***	* * *	* *	
M. purpureus		*****	* *	

(*)'s indicate relative amounts.

The indication that growth of the fungi is possible on the rubber hydrocarbon suggests that in all of the previous experiments the isoprene polymer could have been a source of nourishment.

The writer felt that it would be of added interest to test synthetic rubber for suitability as a nutrient substratum. The E. I. DuPont de Nemours and Company, Inc., furnished samples of the "Plastic Polymer" and of the vulcanized "Du Prene" for

this experimental work. This synthetic rubber is a polymerisation product of chloroprene, comparable to isoprene with the important difference of having the methyl group of isoprene replaced by a chlorine atom.

No growth of Monascus ruber or Monascus purpureus resulted on either the "Plastic Polymer" or the vulcanized "Du Prene" when inoculated and kept for two weeks in a damp chamber at 31° C. The failure of the fungi to grow suggests either that the chlorine atom of the chloroprene unit had an inhibitory effect on growth, or that the natural polymer of isoprene differs from the artificial polymer of chloroprene so that the latter is less liable to attack by the fungi.

DISCUSSION

The relation that the species of *Monascus* may have to the general problem of moulds on rubber in the light of the results of the foregoing experiments is of interest. Since *Monascus* has been reported in the United States, France, South Africa, and China, we may conclude that it is a cosmopolitan genus. In addition to its demonstrated ability to utilize a rubber medium for nutriment, the variety of other substrata on which it has been found bears witness to its use of a great variety of substances. Consideration of these two attributes of *Monascus*, cosmopolitism and utilization of many substrata, leads to the conclusion that *Monascus* may become a conspicuous member of the offending types of fungi found on rubber.

The growths of moulds on rubber have given rubber growers much concern in the past. The chief objection to their presence is the spotting which they produce on the plantation rubber. Sharples (7) reported that such spotting was due to common saprophytic fungi and that most of the offending species belong to the genera *Penicillium* and *Aspergillus*. In a study of spots on crude rubber, Paine (5) observed that a bacterium, *Bacillus prodigiosus*, was responsible for the production of small red spots on crepe rubber. He concluded, however, that the majority of spot discolorations on crêpe was to be attributed to fungi rather than to bacteria, and agreed with Sharples that the most prevalent moulds are *Penicillium* and *Aspergillus*.

It is difficult to find in the literature dealing with fungi growing on rubber any precise information as to the particular species involved. The investigators speak of "light mould growths" and "heavy mould growths," "gray-green mould," and "black and yellow, pin head type of mould," etc. The most complete list found is that given for growths observed on crêpe rubber by Brown (1). The crêpe was supporting growths of Penicillium, Aspergillus, Fusarium, and Cladosporium. The particular species are not named. The present paper establishes the fact that Monascus ruber has been isolated from a mould contamination on crude rubber and that this species and Monascus purpureus can grow on a variety of rubber substrata.

SUMMARY

1. Monascus ruber van Tieghem is reported found at the Botanical Museum of Harvard University growing on a specimen of smoked crude rubber of Hevea brasiliensis attacked by an undetermined species of mite.

2. Both *Monascus ruber* van Tieghem and *Monascus purpureus* Went grew well on the nutriment offered them by the crude rubber apart from any material furnished by the mites.

3. Crude rubbers from various plants served to varying degrees as utilizable media for growth of both species of *Monascus*.

4. Monascus ruber developed somewhat better than Monascus purpureus on unvulcanized samples of commercial rubber: pale crêpe, smoked sheet, and latex-sprayed rubber. Pale crêpe is the least suitable of these as a nutrient substratum. The smoked condition of the smoked sheet seemed to hinder but little the support of the fungi. No growth of either species was produced on the vulcanized samples.

5. Pure rubber hydrocarbon representing the sol-rubber portion of the hydrocarbon constituent supported growths of both species of *Monascus*.

6. Synthetic rubber, a polymer of chloroprene, was not utilizable as a substrate by either *Monascus ruber* or *Monascus purpureus*.

7. Monascus, a cosmopolitan genus found on a variety of substrata may become an important member of the group of fungi attacking rubber.

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CENANGIUM MOLLIUSCULUM

EDITH K. CASH

The taxonomy of this fungus came under consideration in comparing it with a discomycete on *Betula lutea* collected by J. W. Groves at South Aurora, Ontario, and received through the courtesy of H. S. Jackson. The specimen was found to agree with one of *Cenangium molliusculum* Schw. on *Betula carpinifolia* in the Michener Collection at Washington. Later a third collection of the fungus was made by C. L. Shear on *Betula* sp. in the Shenandoah National Park, Virginia.

The species was described by Schweinitz in the Synopsis Fungorum in America boreali, p. 239, 1832, as follows:

- "2008. 31. C. molliusculum, L. v. S., eximia species in Betula carpinifolia Mauch Chunk.
- "C. pezizoideum, aggregatum ac sparsum, saepe invicem adpressum et inde angulatum. Statu madido molliusculum 1-3 lineas latum, nigro olivaceum marginatum. Sicco statu corneo-ceraceum. Sessile, basi tamen contracta. Cupulam refert marginatam subrepandam lobatam, extus olivaceum, disco convexo rugoso, punctato, nigro. Intus substantia eleganter flavo-ferruginea."

The specimen in the Michener Collection on which the following description was based is labeled "in *Betula carpinifol*. Beth. ex Herb. Schw. 2008–31—S§n. Fung." and except for smaller apothecia it agrees very well with the description as published. It should be noted that the locality cited in the description is Mauch Chunk, that on the specimen is Bethlehem, so that it is questionable whether this specimen is the type. However, there is no indication that Schweinitz made more than one collection and there may have been an error in copying the data on the label.

Various characters evident in the specimen indicate that Cenangium molliusculum is a species of Dermatea, rather than Cenangium: notably the small, flat, coriaceous disk, the dark epithecium formed by the branched paraphyses, and the septate spores. As the species, so far as is known, has not been noted in mycological literature since the original publication, a more complete description is given.

Dermatea molliuscula (Schw.) comb. nov.

Syn.: Cenangium molliusculum Schw. Trans. Am. Phil. Soc. II. 4: 239. 1832.

Apothecia sessile, soft when moist, coriaceous when dry, emerging from beneath the bark singly or in groups of 2–3, turbinate to applanate, externally dark brown or slightly olivaceous when moist, hymenium shining black, rough, .5–3 mm. diam., margin brown, distinct, slightly undulate; asci cylindrical-clavate, gradually attenuated toward the base, wall thickened at the apex, 8-spored, 90– 110×11 – $14 \,\mu$; spores narrow ellipsoid, uniseriate below, biseriate above, straight or more generally slightly curved, at first unicellular then 1–3-septate, hyaline to subhyaline (brown in old, disintegrating asci), 20– 23×4 – $7 \,\mu$; paraphyses delicate, filiform, branched toward the tip, at first hyaline, becoming dark and granulose, and coalescing to form a dense, dark brown epithecium; hypothecium thick, pale brownish, plecteachymatic, darker at the cortex.

Specimens examined:

On Betula carpinifolia, Bethlehem, Pa., ex herb. Schw. (Type?). On Betula lutea, S. Aurora, Ontario, Sept. 25, 1934, J. W. Groves 278, Univ. Toronto Crypt. Herb. 6773.

On Betula sp., Shenandoah Nat. Park, Va., along Rapidan River near Hoover Camp, June 26, 1935, C. L. Shear.

BUREAU OF PLANT INDUSTRY, WASHINGTON, D. C.

THE PERFECT STAGE OF BOTRYTIS CONVOLUTA 1

F. L. DRAYTON

(WITH 9 FIGURES)

The possibility of a genetic connection between Botrytis and Sclerotinia was advanced by de Bary (1866). Later, in the revised edition of his book (1884), he again refers to this, stating with assurance that Botrytis cinerea Pers. is the conidial stage of Sclerotinia Fuckeliana (de B.) Fuckel. This statement has been accepted by some mycologists and vigorously rejected by others on the grounds of insufficient evidence. In the early years of this controversy, Ludwig (1892) described Sclerotinia Galanthi, indicating that the apothecia were associated with a Botrytis form on diseased snowdrops, and he assumed a genetic connection. No opinion can be given in this paper on the validity of these early records for they have not been critically examined. It should be pointed out, however, that in this early work the evidence in support of a Botrytis-Sclerotinia connection was based on the association of the two forms on the same substrate, the close resemblance of the disease symptoms, and the similarity of the mycelia and organs of attachment. In the light of our present knowledge, no indisputable case for a genetic connection could be made from observations of this kind.

By means of improved technique, several investigators have demonstrated beyond doubt that the genus *Sclerotinia* exhibits pleomorphism in a number of its species. The first published record of this kind was by Seaver (1917) and Seaver and Horne (1918). These authors established the connection between a *Sclerotinia* and a *Botrytis*, in a fungus attacking the root stocks of *Geranium maculatum*, which they named *Sclerotinia* (*Stromatinia*) *Geranii*. Two years later, Godfrey (1919, 1923), in his work on

¹ Contribution No. 477 from the Division of Botany, Experimental Farms Branch, Department of Agriculture, Ottawa, Canada.

the gray mold of castor bean, established the connection between a Botrytis of the cinerea type and a Sclerotinia, giving to it the name S. Ricini. Another example to which reference may be made is recorded by van Beyma Thoe Kingma (1927), describing a Botrytis isolated from leek seeds which developed apothecia from the sclerotia and in which the conidial stage could be recovered from single ascospore cultures. He named this fungus Sclerotinia Porri.

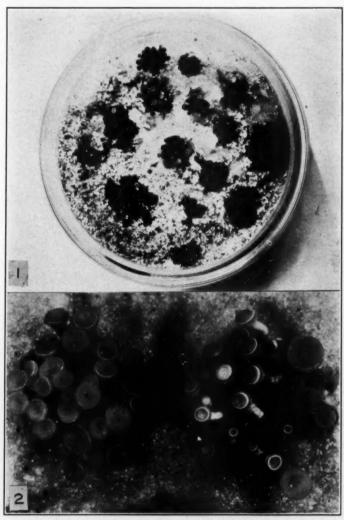
It is now my privilege to record an additional example of a Botrytis-Sclerotinia connection. The fungus is one described by H. H. Whetzel and the author (1932) as the cause of a destructive disease of garden iris which was named Botrytis convoluta. While the conidiophores and conidia of this fungus are those of a Botrytis of the cinerea type, the sclerotial masses with their conspicuously convoluted structure are sufficiently distinctive to have warranted the creation of a new species. Microconidia of the type found in species of Sclerotinia were also noted, it was then predicted that these would function as spermatia in the production of apothecia. Although no apothecia have so far been observed in nature, they have been obtained in the laboratory under carefully controlled conditions, including the use of microconidia for spermatization.

MATERIAL AND TECHNIQUE

The eight isolates used in this investigation were obtained from diseased iris plants originating in a number of localities as follows: S120 from Germany in 1921, B629 from France in 1922, B673 and B1036 from Ithaca, N. Y., in 1924 and 1931 respectively, B927 from a nursery near Ottawa, Ont., in 1928, B1035 and B.c.25 from Yakima, Wash., in 1931, and Ir2 from St. Paul, Minn., in 1934. I am indebted to Professor Whetzel for the first three cultures, to Dr. Freeman Weiss for the Yakima material, and to Miss Dosdall for the isolate from St. Paul. These cultures are identical, except for slight differences in the readiness with which they produce macroconidia and microconidia, and in the quantity and size of the sclerotia produced. For example S120, B629, and B927 produce fewer sclerotia but far more conidiophores than do

the other five, while the latter, and especially B673, form microconidia more quickly and abundantly.

To obtain sclerotia that will later produce apothecia, the most



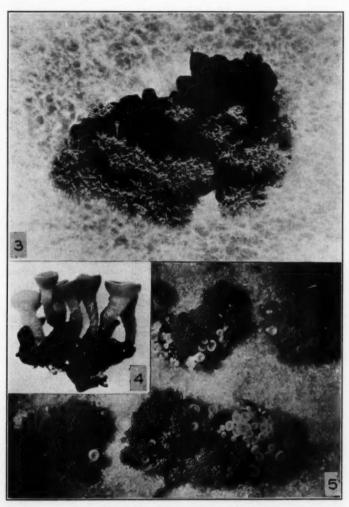
FIGS. 1 AND 2. Sclerotinia convoluta.

favorable substrate is the one used in the studies on *Sclerotinia Gladioli* by Drayton (1934a, 1934b). This is prepared by adding 8 grams of wheat grains and 25 cc. of distilled water to each Petri dish and sterilizing in an autoclave for 30 minutes at 15 pounds pressure.

Varying conditions of temperature, illumination, and the period allowed for vegetative growth and development of sclerotia have been tried. The combination that proved to be most favorable for the subsequent development of apothecia is 14° C. and darkness for a period of 45 days. The sclerotial groups are then removed from these cultures and placed in preparation dishes on moistened quartz sand and kept at 0° C. in darkness for a period of 3 or 4 months. After that they are spermatized with microconidia and put at 5° C. for about 5 weeks. The spermatization is done by preparing a soil extract suspension and applying this to the sclerotia with sterilized camel's hair brushes, as described in the paper on S. Gladioli. During the latter part of the period at 0° C., apothecial fundaments begin to appear and their production is greatly accelerated after the sclerotia are moved to 5° C. When these structures have attained a length of 2 or 3 mm. the dishes are transferred to the greenhouse and placed under cheese cloth covers. There the temperature is held at about 7° C. at night and not exceeding 15° C. during the day. The apothecia attain maturity in about 4 weeks.

It should be noted that the absence of light is specified not only during the 45-day period of vegetative growth but also during the 3 or 4 months when the sclerotia are held at 0° C. If the cultures are subjected to light during the 45-day period at 14° C., the sclerotia, and particularly those of the isolates S120, B629, and B927, become completely covered with conidiophores and conidia after they are put at 0° C., even if kept in darkness at that temperature. In addition, the same result is obtained if the cultures are kept in darkness at 14° C. for 45 days, but with artificial light admitted to the 0° C. chamber while the sclerotia are on the moist sand. In every case where this prodigious development of conidia has occurred, the sclerotia tend to shrivel and usually fail to produce apothecia.

The admittance of light for a few weeks to the 0° C. chamber in one series of cultures resulted in the formation of some conidiophores and apothecia on all of the sclerotia in one dish. Part of



Figs. 3-5. Sclerotinia convoluta.

this dish is illustrated (FIG. 5) and is a striking demonstration of the pleomorphism of this fungus.

THE RECEPTIVE STAGE

In the studies on Sclerotinia Gladioli it was shown that under favorable treatment, receptive bodies develop from a stroma and that at this stage fertilization and the resulting apothecial development could be brought about by spermatizing with microconidia from a compatible isolate. In the case of the fungus here described, great difficulty has been experienced in determining when the spermatization should be done, for no distinctive receptive structures as such have been recognized. In the absence of a structural indication of this critical stage, three possibilities present themselves. Fertilization may occur when the sclerotia are forming; small groups of trichogynous hyphae may protrude through the rind of the mature sclerotia; or the apothecial initials, which emerge after the period of rest at 0° C., may constitute the receptive structures.

The first hypothesis was tested by a large series of cultures in which spermatization was begun on the 15th day after inoculation, at the first sign of sclerotial development. This was repeated at two-day intervals up to the 25th day. The sclerotia were removed on the 35th day and subjected to the optimum conditions outlined above. No apothecia developed in these cultures, so it appears that this hypothesis is to be discarded. In order to test the second possibility, large numbers of microtome sections of mature sclerotia were cut and stained. These failed to reveal any trace of ascogonial coils or of protruding trichogynous hyphae that might be connected with such coils. That seems to exclude the second possibility. Series of sections made at later dates, however, provided some evidence in favor of the third hypothesis. Sections of sclerotia that had been kept at 0° C. for 4 months showed distinct groups of specialized hyphae just beneath the rind. When it was found that the apothecial initials emerged at a point directly over these hyphae, it seemed likely that they are part of the ascogonial system. It is probable that fertilization cannot take place in the absence of an ascogonium, so it is suggested that the protruding apothecial initial is, in the early stages of its development, the

receptive structure. The fact that in an early culture series apothecia were obtained in a few dishes, only after respermatization towards the close of the dormancy of the sclerotia, lends strong color to the above theory and provides an explanation for the lack of uniformity in the behaviour of the earlier culture series.

The apothecia illustrated in this paper and used as type material for the description of this ascigerous stage were obtained from only a few cultures in the earlier experiments even though many dishes contained the same isolate and were spermatized with the same microconidia. It is impossible at this time, therefore, to give any definite information on the sexual interaction of the isolates used. This and other facts about the sexual mechanism are being investigated.

It is of interest, however, to point out the apparent reason for this failure to obtain more consistent development of apothecia. In the older series, spermatization was carried out just after the mature sclerotia were placed on moist sand and prior to placing them at 0° C. Assuming that the receptive stage occurs when the ascogonial initials emerge from the sclerotia, this early spermatization meant that the microconidia would have to survive the intervening 4 or 5 months at 0° C. in order to effect fertilization. mucilaginous substance in which the microconidia are borne presumably aids materially in keeping these spores alive for long periods, but in the laboratory process of spermatization, the microconidia are applied as a suspension in soil extract and a great deal of this protective coating is dissolved. It seems likely that in most dishes the microconidia would become inactivated after 4 or 5 months, but that in a few dishes where moisture conditions happened to be more favorable, the microconidia would survive and fertilize the initials. In the more recent cultures, spermatization has been done at the later stage, as outlined in the section on technique, and one of these series is sufficiently advanced now to predict a more uniform production of apothecia.

THE GENETIC CONNECTION

In the cultures that yield apothecia, a great many fruiting bodies are produced from each sclerotial group (FIG. 1, 2)—as many as

30 have been counted on a single agglomeration. The apothecia possess all of the morphological features that characterize the genus *Sclerotinia*, but since a detailed description is given in the emended technical description, it need not be repeated here.

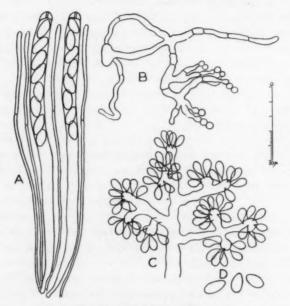


Fig. 6. Sclerotinia convoluta.

Ascospores from the apothecia were shot on potato-dextrose agar in Petri dishes. They germinated in 4 or 5 hours and 40 single-spore and several multiple-spore cultures were made. The former are identical with the original isolates. Conidiophores, conidia, and the characteristic convoluted sclerotial masses appeared on all of the cultures. A somewhat different appearance was noted in the multiple-spore cultures and it is of interest to speculate on the reason for this. In the original isolates which were obtained from plantings of conidia or sclerotia and those grown from single ascospores, both the aerial and sub-surface mycelium is white and the sclerotia are produced in abundance. The mass ascospore cultures are decidedly less vigorous, the

amount of aerial mycelium and the number and size of the sclerotia are greatly reduced, but the most striking differences are the brown color of the sub-surface mycelium and the early development of a great abundance of microconidia.

When certain pairs of the original isolates or those from single ascospores are grown on Petri dishes of potato-dextrose agar a



Figs. 7-9. Sclerotinia convoluta.

marked brown line from 1 to 3 mm. across appears at the junction line and in and on this line large numbers of microconidial sporodochia appear (FIG. 9). The significance of this reaction is not understood, but it is apparently indicative of some form of antagonism between these thalli. In view of this, the peculiar characters exhibited by the multiple-spore cultures would seem to be the resultant of a great many reactions comparable to the junction line behavior with complete diffusion of the brown subsurface mycelium and the presence of microconidia all over the surface of the slanted agar. Quite apart from the academic interest, this phenomenon indicates the necessity of using single ascospore cultures to describe cultural characters accurately.

Many of the single spore cultures have been grown on wheat, spermatized, and kept under conditions conducive to apothecial production. At the time of writing, abundant apothecial fundaments have developed from the sclerotia, but the apothecia are not yet mature.

EMENDED TECHNICAL DESCRIPTION

The development of apothecia by the fungus previously described as *Botrytis convoluta* Whetzel and Drayton, now makes it possible to complete the technical description, as follows:

Sclerotinia convoluta sp. nov.

Synonymy—Botrytis convoluta Whetz. & Drayton, Mycologia 24: 475. 1932.

Apothecia densely gregarious, arising from the sclerotial agglomerations (FIG. 2), infundibuliform to cyathiform becoming discoid, hymenium snuff brown, under side of cup and the stipe sayal brown (Ridgway), with a narrow, lighter band around the edge of the disc, sterile portion prosenchymatous with a definite hypothecium (FIG. 7); stipitate, varying in height from 3–6.25 mm., discs 2.5–4.0 mm. in diameter, stipes with more or less pronounced narrow, spiral, fibrillose ridges extending to the base of the cup (FIG. 4), the outer surface of the cup apparently glabrous, but on drying becoming subtomentose especially at the edge of the disc. Asci cylindrical, $150-195 \times 9-13 \,\mu$, the plug in the thickened apex staining blue with iodine (FIG. 6A). Ascospores 8, occupying $50-95 \,\mu$ of the ascus, uniseriate, ellipsoid, hyaline,

continuous, nonguttulate, uninucleate when first delimited (FIG. 8), at maturity with 2 or 4 nuclei, 11.7– 19.5×5.2 – $9.1 \,\mu$, average $14.87 \times 6.9 \,\mu$, mode 14.3– 15.6×6.5 – $7.8 \,\mu$. Paraphyses abundant, filiform, septate, hyaline, 2.5– $3 \,\mu$ in diameter, occasionally wider near the apex.

Mycelium profusely branching, hyaline, becoming tan-colored with age at the surface of the substrate, multinucleate, $4.5-7.5 \mu$ in diameter.

Sclerotia shining black, convolute-agglomerated (FIG. 3), up to 18×16 mm. in size, frequently hollow in the centre, with a distinctly differentiated, black, pseudoparenchymatous rind, and a white medulla composed of more or less loosely intertwined hyphae, with slightly thickened walls, embedded in a colorless, homogeneous matrix.

Conidiophores brown, erect, fasciculate, branched at the apex, about 1 mm. tall, $9-12 \mu$ in diameter at the base, tapering toward the apex, arising from large, dark, thick-walled cells in the mycelium or from medullary cells just beneath the rind of the sclerotium (Fig. 3).

Conidia light brown, one-celled, smooth, ovate to slightly pyriform, borne in dense clusters on sterigmata produced from the swollen ampullae of the ultimate branchlets of the conidiophores (Fig. 6c); size variable, living spores from diseased rhizomes range from $7-18 \times 5.25-12.75~\mu$, mode $11.0-11.75 \times 9.0-9.75~\mu$, average $11.41 \times 9.25~\mu$ (Fig. 6d); somewhat smaller when produced on culture media. Cultures bearing conidia emit a sweetish aromatic odor.

Microconidia globose, $4-4.5 \mu$ in diameter, uninucleate, produced on a sporodochium made up of closely septate hyphae that give rise to numerous clusters of verticillately branched conidiophores ending in tapering, elongate, terminal cells (phialides) on which the microconidia are developed in vast numbers, embedded in a mucilaginous matrix which on drying, gives a waxy consistency to the whole sporodochium; produced on the mycelium, on the sclerotia, or by ascospores germinated in soil extract or water (FIG. 6B).

The cause of a necrotic disease of the garden iris, known as *Botrytis* rhizome rot. Sclerotia and conidia found in early spring (March and April); apothecia unknown in nature. Known from the United States, Canada, Germany, France, Holland, and England.

Apotheciis e sclerotiis orientibus, infundibuliformibus usque ad cyathiformibus, formam disci assumentibus, parte sterili prosenchymatosa, stipitata, 3–6.25 mm. alta; discis 2.5–4 mm. in diametro. Ascis cylindricis, 150–195 \times 9–13 μ . Ascosporis 8, uniseriatis, ellipsoideis, hyalinis, primo uninucleatis,

sed postea 2 aut 4 nucleos habentibus, 11.7–19.5 × 5.2–9.1 μ , modulo 14.3–15.6 × 6.5–7.8 μ .

Sclerotiis atro-nitentibus convolutis agglutinatis, usque ad 18×16 mm.; conidiophoris brunneis, erectis, fasciculatis apice ramosis, circa 1 mm. altis, e cellulis callosis mycelii aut e sclerotiis orientibus; condiis pallide brunneis, ovatis vel pyriformibus, 7– $18 \,\mu$ longis, 5.25– $12.75 \,\mu$ in diametro; microconidiis globosis, 4– $4.5 \,\mu$ in diametro.

In hortis irides necat.

Type specimens of the conidial stage deposited in the Plant Pathological Herbarium, Cornell University, Ithaca, N. Y., No. 12615. Type specimens of the apothecial stage deposited in the Herbarium, Division of Botany, Central Experimental Farm, Ottawa, Ont., No. 3010, the Plant Pathological Herbarium, Cornell University, Ithaca, N. Y., No. 25282. Duplicate material from the same cultures also deposited in the Farlow Herbarium, Harvard University, Cambridge, Mass., The New York Botanical Garden, New York, N. Y., the Royal Botanic Gardens, Kew, Surrey, England, the Pathological and Mycological Collections, Bureau of Plant Industry, Washington, D. C., and the Department of Botany, University of Toronto, Toronto, Ont.

SUMMARY

Another instance of a genetic connection between species of the genera *Botrytis* and *Sclerotinia* is recorded. The fungus is one causing the *Botrytis* rhizome rot of garden iris described by Whetzel and Drayton and named *Botrytis convoluta*.

Under carefully controlled cultural conditions, combined with the use of microconidia for spermatization, the convoluted sclerotial masses of this fungus have developed apothecia of the *Sclerotinia* type. The connection of this ascigerous stage with the conidiophores and conidia of the imperfect stage is established.

The new binomial *Sclerotinia convoluta* is proposed and a technical description is given.

ACKNOWLEDGMENTS

In the preparation of the histological material and the taking of some of the photographs, the assistance of Dr. R. E. Fitzpatrick, formerly graduate assistant in this laboratory, is gratefully acknowledged. The rest of the photographs were taken by Mr. A. J. Hicks and Dr. J. W. Groves. The author is also indebted to Professor H. H. Whetzel for his unfailing interest and cooperation during the course of this investigation.

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EXPLANATION OF FIGURES

- Fig. 1. A preparation dish with sclerotial groups bearing apothecia. Natural size.
 - Fig. 2. Two of the above groups of apothecia $(\times 6)$.

atous iris. Mycologia 24: 469-476. 1932.

- Fig. 3. A convoluted sclerotial mass bearing conidiophores and conidia $(\times 6)$.
- Fig. 4. A side view of a group of apothecia showing the spiral fibrillose markings on the stipes $(\times 8)$.
- Fig. 5. Four groups of sclerotia bearing conidiophores and apothecia $(\times 5)$.
- Fig. 6. A, Asci, ascospores, and paraphyses. B, An ascospore germinating in soil extract and producing microconidia. C, Conidiophore with immature conidia. D, Mature conidia.

Fig. 7. A longitudinal section of an apothecium stained with gentian violet and safranin. Note the young and mature asci, the hypothecium, and the prosenchymatous context $(\times 40)$.

Fig. 8. Portion of a longitudinal section of an apothecium. One ascus shows the uninucleated condition of the ascospores when first cut out, and each of the other two asci with a fusion nucleus (\times 850).

Fig. 9. Portion of the junction line of a culture from paired isolates, showing the development of surface and sub-surface microconidial sporodochia $(\times 6)$.

d

MIMICRY IN HYPOXYLON

WILLIAM W. DIEHL

(WITH 3 FIGURES)

The accompanying illustration (FIG. 1–3) shows an abortive development of *Hypoxylon marginatum* (Schw.) Berk., accompanying the characteristic plant. Each abnormal stromatic unit possesses most strikingly, but entirely superficially, the appearance of *Camillea Sagraeana* (Mont.) Berk. & Curt. These abnormal stromata are sterile and without any trace of perithecia. No parasite was definitely evident, and no explanation is suggested for this condition. The specimen was found by Dr. C. L. Shear near Falls Church, Va., Oct. 8, 1925.

Two similar specimens, gathered in 1928 by R. Kent Beattie (nos. 62 and 67) at Kuraru, in Taiwan (Formosa), have lately been available for comparison. Most of the stromata in this material show the *Camillea*-like appearance as in the specimens from Virginia. A few, however, are more nearly normal, although without asci or spores. These resemble *Hypoxylon cohaerens* Fries, to which species these Formosan specimens are probably referable.

The resemblance to a species of the characteristically tropical genus Camillea shown by these aberrant specimens of two temperate-zone species of Hypoxylon is so striking that one familiar with both genera could readily be deceived by superficial examination only. Although they are teratological manifestations of two different species of Hypoxylon it is remarkable that they simulate a distinctive species of the related genus Camillea.

To call such a resemblance "mimicry," although the term has been practically unused in mycology, is to be condoned perhaps by its frequent and time-honored usage for such a condition in organisms which possess comparable resemblances no more expressive of volition than in fungi.

DIVISION OF MYCOLOGY AND DISEASE SURVEY, BUREAU OF PLANT INDUSTRY, WASHINGTON, D. C.

¹ Cooke, M. C. Mimicry in fungi. Grevillea 9: 151-153. June 1881.

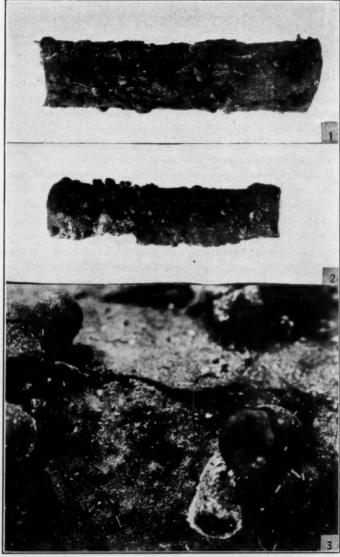


Fig. 1, chiefly normal stromata (\times 1); 2, abnormal and normal stromata (\times 1); 3, abnormal stromata (\times 10). Photographs by Mr. M. L. S. Foubert.

A NEW SPECIES OF DOTHIORELLA CAUSING DIE-BACK OF ELM

A. F. VERRALL AND CURTIS MAY 1

(WITH 6 FIGURES)

Dothiorella Ulmi is the pycnidial form of the fungus previously reported as causing the disease commonly known as the Cephalosporium die-back of elm. This disease was observed on American elm (Ulmus americana L.) in Minnesota in 1929 and the causal organism tentatively placed in the Sphaeropsidales because of pycnidia formed on malt agar (5). The same organism was isolated from specimens collected in 1930 in the midwestern and eastern states and reported as Cephalosporium sp. (3). The fungus has also been reported from Nebraska (2). The disease of American elm noted from Connecticut in 1930 (1) has subsequently been found by a comparison of cultures to be caused by the same organism.

The Cephalosporium stage of the fungus was isolated from diseased elms. Transfers were made from these cultures to sterilized elm twigs upon which pycnidia developed abundantly. Similar pycnidia have been found on naturally diseased elm. Single-spore isolations from pycnidia on specimens collected in New Jersey and Virginia produced the typical Cephalosporium stage on agar. Trees inoculated with single-spore isolates developed typical symptoms of the disease and both stages of the fungus were recovered from them.

The characteristics of the pycnidial stage of the fungus place it in the genus *Dothiorella* in the broad sense as used by Saccardo

¹ Respectively, Assistant Pathologist, Division of Forest Pathology, and Emergency Conservation Work, and Senior Pathologist, Division of Forest Pathology, Bureau of Plant Industry, U. S. Department of Agriculture.

Acknowledgment is given R. U. Swingle and L. M. Fenner of the Division of Forest Pathology, C. L. Shear of the Division of Mycology and Disease Survey, U. S. Department of Agriculture, and the Staff of The New York Botanical Gardens for assistance, criticisms, and use of herbarium material.

(4). A search of literature and an examination of available herbarium specimens and a comparison of cultures of various species of *Cephalosporium* failed to disclose any *Dothiorella* or *Cephalosporium* identical with the die-back organism. The fungus is here described as a new species of *Dothiorella*.

Dothiorella Ulmi sp. nov.

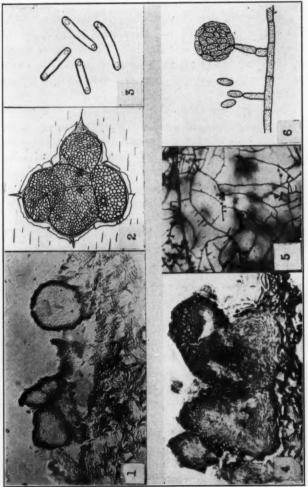
Stroma basal (FIG. 1), irregularly circular to elongate, 100–385 μ across, subepidermal, early erumpent; pycnidia partially imbedded in the stroma (FIG. 1, 2), in groups of 2–12, occasionally single, black, at first solid and subsclerotoid, and finally with a more or less subcoriaceous wall, glabrous, globose to irregular, 63–161 μ in diameter, occasionally reduced to irregular chambers (FIG. 4); ostioles non-papillate, 7–13 μ in diameter; conidia (FIG. 3) 1-celled, hyaline, elongate, rounded at both ends, straight or occasionally slightly curved, 2.9–5.4 \times 0.5–1.0 μ (av. 3.6–0.8 μ); conidiophores absent, the conidia being histogenic.

Stroma basilare, irregulariter orbiculatum vel elongatum, in diametro 100–385 μ , subepidermale, erumpente; pycnidia in stromate subimmersa, 2–12 aggregata, aliquando singularia, atra. glabrata, globosa vel irregularia, in diametro 63–161 μ , aliquando ad loculos irregularos reducta; ostiolo epaillati, in diametro 7–13 μ ; conidia 1-cellularia, hyalina, elongata, utrinque rotundata, recta vel nonumquam leniter curvata, 2.9–5.4 \times 0.5–1.0 μ (av. 3.6 \times 0.8 μ); conidiophori nulli.²

This species is characterized by inconspicuous pycnidial groups, small pycnidia, and by small, histogenic spores. Type material has been deposited in the mycological collections of the Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C., under No. 70804.

Occurring on elm (Ulmus americana L. and U. fulva Michx.) as a cause of a prevalent die-back characterized by brownish discoloration of the cambial region and outer layers of wood, wilting of leaves, and cankers, at first reddish brown, then becoming darker. Pycnidia develop sparsely on newly formed cankers on twigs and small branches and are often associated with Sphaeropsis, Phoma, Cytospora, and other fungi as the cankers age. Pycnidia have been found on elm collected in Virginia, Connecticut, Ohio, New Jersey, and Oklahoma.

The die-back fungus has been isolated from 42 per cent of the ² Latin description by R. Kent Beattie, Division of Forest Path., U. S. Department of Agriculture.



Figs. 1-6. Dothiorella Ulmi.

57,547 specimens of elm submitted from 1930 to 1935 as suspected of having the Dutch elm disease. Most of these specimens were supposedly American elm but a few from which the die-back organism was isolated were slippery elm (*U. fulva* Michx.). The die-back fungus was isolated from specimens collected in 28 states, the District of Columbia, and Canada. The disease is common where elms occur from Virginia to Oklahoma northwards to Canada. A few infected specimens have been received from Colorado and Montana.

Cultures of D. Ulmi on malt or potato-dextrose agar are very variable in color and rate of growth. Usually cultures are brown, slow growing, with a filamentose margin, frequently forming elongate yellow crystals in the agar below the mat, and producing a sweet aromatic odor. Cephalosporium heads (FIG. 5, 6) develop in isolated groups on the mycelium. The conidiophores are relatively short compared with the described species of Cephalosporium, being 0.7–20 μ (av. 5.6 μ) in length; straight, mostly unbranched. Conidia are hyaline, 1-celled, elliptic, 4.5 \times 1.9 μ . Pycnidia sometimes develop singly or in small groups in agar. The pycnidiospores are identical to these formed in nature.

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EXPLANATION OF FIGURES

Fig. 1-6. Dothiorella Ulmi: 1. Section through stroma and pycnidia in bark. $130 \times .2$. Camera lucida drawing of pycnidial group showing ostioles and arrangement on stroma. $100 \times .3$. Camera lucida drawing of spores from a pycnidium. $3900 \times .4$. Section through pycnidia showing disappearance of side walls forming an irregular chamber. The pycnidia are filled with the pseudotissue from which spores are formed. $250 \times .5$. Mycelium in culture showing aggregations of Cephalosporium heads. $100 \times .6$. Camera lucida drawing of Cephalosporium heads in culture. $1000 \times .6$.

A NEW SPECIES OF TUBERACEAE FOR AMERICA

CLOYD BURNLEY STIFLER

On August 26, 1930, specimens of *Hydnotria carnea* Corda were found by the writer, half-buried in the mud, in a path beside the Lower Tunkhanna, a trout stream near Fern Ridge in the Pocono Mountains, Pennsylvania.

Two small potato-like fruit bodies were collected and preserved in 70 per cent alcohol containing a little glycerine.

When examined microscopically they proved to be hypogaeous ascomycetes and were identified by the writer as either *Hydnotria Tulasnei* Berk. & Br., or *Hydnotria carnea* Corda according to the descriptions in Hesse's *Die Hypogaeen Deutschlands*.

The fruit bodies were irregularly globose and the size of hickory nuts $(2.7 \times 2 \text{ cm.})$ and $2.3 \times 1.7 \text{ cm.})$. They are nearly smooth on the exterior surface with a few small fissures or depressions. The color is between flesh and a rusty brown. To the unaided eye there is no distinct peridium. Any slight floccosity may have been lost when the mud was removed. Small canals present in the gleba are lined with the hymenium. The asci are generally long stiped and cylindrical although some are clavate. In size they average 220–34 μ (in upper portion).

The spores are usually uniscriate but occasionally biseriate near the middle of the ascus which then appears to bulge on one side at that point.

The number of spores varies. Usually 8 are present but in some cases fewer than that. They are irregularly globose. The exposure is composed of thick blunt warts and varies in color from yellow to dark brown. The size varies from 31–36 \times 34–37 μ including the warts. The exospore varies from 2.5 to 7.7 μ in thickness due to these warts.

A few asci lie at the base of the others and are perpendicular to them. The paraphyses are colorless; irregularly cylindrical, septate, much longer than the asci and about $6.8\,\mu$ in diameter.

The banks along the Little Tunkhanna are suitable for the

growth of various fungi. It bordered by rhododendron. The trees are beech, grey birch, maple and hemlock. The ground cover is composed of mosses, arbutus, partridge berry and lichens, and in a few pots some grasses.

Hesse states that the species *Hydnotria carnea* occurs in Europe in sheep pastures, under trees, where sheep find shade at midday and where their excrement, trampled into the soil yields a rich compost, and that when ripe the fruit bodies work up toward the surface until they are partly exposed.

There are no sheep in this locality but deer are plentiful and deer dung may have been trampled into the soil, however, there is no evidence of this.

The place where the fruit bodies were found has been visited each summer since 1930 but no more specimens have appeared.

A section of the largest fruit body was sent to Dr. Fred J. Seaver who agreed that the specimen was a *Hydnotria*, probably *Tulasnei*, and suggested that it was new to America and should be referred to Dr. Helen M. Gilkey for identification.

After examining it Dr. Gilkey wrote that there had been some confusion between the two species, that Tulasne considered Hydnotria carnea a synonym for H. Tulasnei, and Dr. Ed. Fischer was undecided as to whether they might be two varieties of one species —Hydnotria Tulasnei—and stated, "Your specimen is characterized by a cylindrical rather than club shaped ascus. The gleba is not as dark brown as it is sometimes figured, but that may be due to the fact that the material is not completely mature though enough spores are ripe to leave no question concerning their ultimate form. I am very pleased at this first record of this species in America."

A post script written after she had read further notes by Dr. Ed. Fischer, however, states, "Your specimen is *Hydnotria carnea* Corda. At any rate this is the first report of it from America to my knowledge."

A letter just now received, January 1937, from Dr. John A. Stevenson states that, "As far as Miss Cash and I have been able to determine *Hydnotria carnea* Corda has not been found in this country."

CHICAGO, ILL.

SPORULATION OF THE PHIALOPHORA TYPE IN HORMODENDRUM

C. W. EMMONS AND A. L. CARRIÓN

(WITH 6 FIGURES)

Hormodendrum is an important genus of the Fungi Imperfecti. Its species have a wide geographical distribution and are commonly present in soil and decaying vegetation. Because of the ubiquity of its wind-blown spores it is a familiar "culture weed" of the laboratory. Most species of the genus are saprophytes, but at least two are facultative pathogens of man and there cause a skin disease known as "dermatitis verrucosa" (chromoblastomycosis). This disease apparently is not contagious, and the evidence at hand indicates that the fungus is a wound parasite capable of growing in animal tissue only after its introduction upon some foreign body such as a thorn. In this respect it is like some of the other mycotic diseases in which the parasitic agent requires certain special, and in some cases, unknown conditions before it becomes pathogenic, but once established in the human body, it is extremely difficult to dislodge.

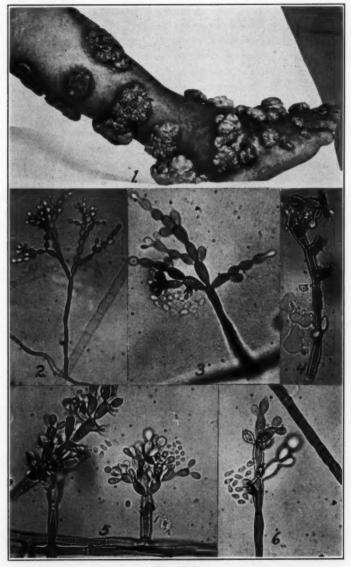
Dermatitis verrucosa occurs chiefly in the tropics, and more rarely in temperate climates. Three cases have been reported from the United States. The disease is generally characterized in man by the formation on an extremity, usually a leg, of warty or cauliflower-like outgrowths of the skin (FIG. 1). In some cases it has been possible to obtain from the patient a history of some slight injury to the foot, followed by the slow appearance and development of a small initial lesion. By slow extension of this primary lesion the entire lower leg may become involved. Extension of the pathologic process is not rapid, and a duration of several years, without metastatic spread, and without serious interference with the activities of the patient is the rule. Sections of the infected tissue show the fungus present in the form of subspherical pigmented cells often occurring in pairs or short chains. In spite of

its benign course, the infection is very difficult to eradicate by the methods of treatment now in general use.

It should be stated that notwithstanding the similarities in clinical aspect of the reported cases of "dermatitis verrucosa" there is some evidence that deviations from the usual clinical type may occur. However that may be, we do know that the disease may be caused by any one of three species of related fungi. The first reported case, which was from Boston, was caused by Phialophora verrucosa.8 studied and named by Thaxter. The most common cause of the disease in Puerto Rico and Brazil is Hormodendrum pedrosoi 4 Brumpt. H. compactum 1, 2 Carrión has been isolated from a single case in Puerto Rico. H. Langeroni 6 was the etiologic agent of a different disease. Recently Moore, 9 without any accompanying definitions, proposed two new generic names for the fungi of "dermatitis verrucosa," and one new species. He very kindly sent us a culture of the latter. The fungus which he named as a new species, Phialoconidiophora guggenheimia, is clearly a typical strain of H. pedrosoi, and the new name is to be added to the already too long synonymy of that species.

Phialophora verrucosa and Hormodendrum pedrosoi have long been known as the commonest causes of the disease. Phialophora verrucosa is characterized by the formation of spores in the mouth of a flask-shaped conidiophore. This may be looked upon as a semi-endogenous type of sporulation. The spores are budded out serially from the base of the cup which terminates this conidiophore and they collect in a droplet at the mouth of the cup. The conidiophore is not capable of further growth, although in abnormal cases sporulation may be interrupted by the formation of a cell, which, instead of being discharged as a spore, remains attached in the base of the flaring cup which forms the mouth of the conidiophore. This cell in turn bursts at the tip, the cell wall at the point of rupture grows into a cup, and this secondary conidiophore then

Fig. 1, skin lesions in a case of *Dermatitis verrucosa* caused by *Hormodendrum pedrosoi*; 2, a well developed conidiophore of the *Hormodendrum* type in *H. pedrosoi* (× about 250); 3, a conidiophore of *H. pedrosoi* in which one cell in the spore chain has produced a *Phialophora*-like cup and spores (× 900); 4, sporulation of the *Phialophora* type in *H. pedrosoi* (× 900); 5 and 6, sporulation of the reduced *Hormodendrum* type closely associated with sporulation of the *Phialophora* type in *H. pedrosoi* (× 900).



Figs. 1-6.

carries on spore production in the usual manner. A few conidiophores open by more than one mouth.

In contrast to the above, *Hormodendrum* is characterized by a type of exogenous spore formation in which the conidia are borne in branching chains which arise from somewhat specialized conidiophores (Fig. 2). Sporulation takes place by a process of budding at the tips of the conidial chains, and the chains are dendroidal or branched because some of the spores bud at more than one point. The mature spore head therefore assumes a tree-like form in which the youngest spores are at the tips of the branches, and, given proper conditions of youth and nutrition, are capable of the production of additional secondary spores. This process may be indefinitely repeated.

Despite their widely separated positions in the Saccardo classification of the Fungi Imperfecti, a genetic relationship between Phialophora and Hormodendrum pedrosoi has long been suspected. Weidman 10 suggested that they might be different phases of the same fungus. Proof of such a relationship, however, had not been given. It was therefore gratifying to be able to demonstrate, during a critical comparative study of strains isolated in Puerto Rico and in other parts of the world, that the type of sporulation characteristic of Phialophora does actually occur in Hormodendrum. This fact was demonstrated for all our collected strains of H. pedrosoi, for H. compactum, and for a saprophytic strain of Hormodendrum isolated in the laboratory. This discovery was reported in 1935 in a preliminary paper 3 in the Puerto Rico Journal of Public Health and Tropical Medicine, and in later papers 2, 4, 5 in the same journal. Conant 7 subsequently found Phialophora sporulation in the strain of H. pedrosoi isolated in North Carolina, and Moore 9 observed it in the strain he reported.

Sporulation in the species *Hormodendrum pedrosoi* is somewhat reduced in comparison with that of saprophytic species of the genus. Most of the spores are produced either in branching conidial chains which are short, but are otherwise characteristic of *Hormodendrum*, or upon conidiophores of a type somewhat resembling those of *Acrotheca*. Conidiophores of the *Phialophora* type occur only rarely, but when found they are well developed and are similar in every way to those of *Phialophora verrucosa* (FIGS.

3–6). Besides their rarity they are somewhat more variable in size and shape than the corresponding structures in *Phialophora*, and they appear to be less prolific. That they are homologous structures in the several species under consideration seems evident, however. In *Hormodendrum* they can be conveniently demonstrated upon cornmeal agar slide cultures. Conidiophores of this type may be grouped together on certain hyphae (FIG. 4), they may be solitary, or they may be formed in very close proximity to the *Hormodendrum* type of conidiophore. The closest possible association of the two types of sporulation occurs when, as sometimes happens, a *Hormodendrum* spore in an otherwise normal spore head becomes transformed by the rupture of the end wall into a conidiophore of the *Phialophora* type (FIGS. 3, 5, 6). This has been seen more frequently in *H. compactum* than in other species.

Observations of stages in the transformation of a *Hormodendrum* spore directly into a conidiophore of the *Phialophora* type gives us some insight into the probable relationship between these two divergent methods of sporulation. We may assume that a given cell appearing as a side branch on the mycelium of *H. pedrosoi*, for example, has the potentialities of either a *Hormodendrum* or a *Phialophora* conidiophore. The predominant tendency is toward the former, but unknown forces may tip the scale toward the latter. When a *Phialophora* conidiophore appears as an integral part of a *Hormodendrum* spore head it means that this plasticity has been retained to a period in development long after morphological differentiation has been well established.

The actual changes which bring about this transformation seem to be as follows. The wall at the tip of one of the spores in an otherwise normal spore head ruptures at the point where it would normally produce a secondary spore. A plasma membrane and a secondary wall retain the protoplasm of this cell, and the broken portions of the ruptured wall develop into a cup, characteristic of *Phialophora*, from the base of which the spores are budded out. It may well be that *Phialophora verrucosa* is a mutant which arose from some species of *Hormodendrum* when this tendency to cup formation became dominant over secondary budding.

The possibility that these anomalous spores, appearing infrequently on the mycelium of *Hormodendrum*, are not conidia but are actually spermatia, is obviously to be considered. The bottle-shaped sporophore which bears them undoubtedly resembles a spermatiophore. The spores, which are about $1.5-2\times2-3~\mu$, might well be spermatia. In view, however, of our belief that both sporophore and spore are homologous with the corresponding structures which constitute the only known method of reproduction in *Phialophora*, and in the absence of any demonstrated spermatial function for these spores, we prefer to look upon them as conidia. The spores of *Phialophora* germinate readily by a sort of pseudobudding, but this, as Dodge has shown for other fungi, can not be taken as proof of a non-spermatial character. If they be spermatia, however, they now function perfectly in *Phialophora* as conidia.

If, as certainly seems possible, the spores of the *Phialophora* type developing infrequently in cultures of *Hormodendrum pedrosoi* and *H. compactum* are actually spermatia, it is to be hoped that their discovery foreshadows the demonstration of an ascomycetous phase of these fungi. In view of this and of other considerations it is deemed advisable to retain for these fungi the generic name *Hormodendrum* under which they were first described, rather than transfer them to *Cladosporium*.

The production of two types of conidia by one fungus is by no means unknown to mycologists. In this case, however, sporulation of a semi-endogenous type and exogenous sporulation, occurring either on different parts of the same mycelium or in such close association that one is derived directly from the other seems noteworthy. This association seems particularly significant in this case because it indicates a relationship, previously only suspected, between two etiologic agents of one disease.

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PHOTOGRAPHS AND DESCRIPTIONS OF CUP-FUNGI—XXVII. PEZICULA ON CORNUS 1

FRED J. SEAVER

(WITH 2 FIGURES)

The writer recently received specimens of *Pezicula* on *Cornus* collected at East Hampton, New York, which was at first thought to be *Pezicula Corni* Petrak. However, critical study showed it to be quite different in spore characters from that species. Since the New York species varies greatly from *Pezicula Corni*, which has been collected from California to Oregon, we take this opportunity to describe it as a new species, publishing illustrations of both in order to show the differences.

Both of these species are associated with a *Myxosporium* which appears to be respectively their conidial stages. Attempts to culture the material from New York were unsuccessful, owing to the fact that the spores had apparently been killed by the disinfectant. The New York material was sent to the writer by R. P. White who has done some culture work which indicates that the *Myxosporium* is the conidial stage of the new species. The writer will be glad to receive any other material of this genus from this host.

Pezicula Corni Petr. Ann. Myc. 20: 197. 1922. ?Dermatea Corni Phill. & Hark. Grevillea 13: 22. 1884. Pezicula rhabarbina f. Corni Ellis in Herb.

Apothecia solitary or cespitose, at first rounded, becoming expanded and subdiscoid, with a mealy brown covering, reaching a diameter of about .5–1 mm.; hymenium plane or nearly so, yellowish to dark brownish-black; asci clavate, reaching a length of 120μ and a diameter of 27μ , 8-spored; spores ellipsoid irregularly

¹ This paper is preliminary to a monograph of North American Cup-fungi (inoperculates), a companion volume to North American Cup-fungi (operculates), which was published by the author and issued in December, 1928.

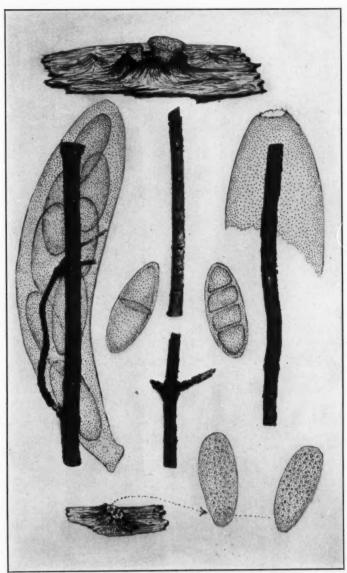


Fig. 1. Pezicula Corni.

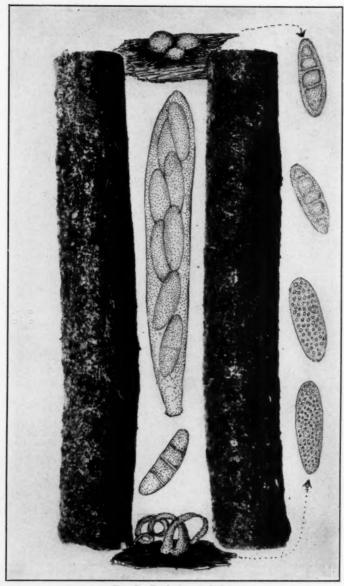


Fig. 2. Pezicula cornicola.

SEAVER: CUP-FUNGI-XXVII. PEZICULA ON CORNUS 337

crowded in the ascus, 10–13 \times 28–34 $\mu;$ paraphyses filiform, slightly enlarged above.

On Cornus alternifolia, C. stolonifera, and unnamed species of Cornus.

TYPE LOCALITY: Idaho.

DISTRIBUTION: California to Oregon and Ontario.

Exsiccati: N. Am. Fungi 2809 (as Pezicula rhabarbina).

This is accompanied by a Myxosporium which appears to be its conidial stage. The pycnospores are ellipsoid and densely filled with granules, $13-15 \times 33-36 \mu$.

The identity of the Phillips and Harkness species is uncertain.

Pezicula cornicola sp. nov.

Apothecia usually in cespitose clusters, individual apothecia sessile reaching 1 mm. in diameter, pale yellow; hymenium plane or slightly convex; asci clavate, reaching a length of 100–120 μ and a diameter of 12–15 μ ; spores partially 2-seriate, ellipsoid, straight or slightly curved, about 7–8 \times 20 μ , becoming tardily 1–3-septate; paraphyses filiform, slightly enlarged at their apices.

Apotheciis cespitosis, sessilibus 1 mm. diam. dilute-flavibus; disco planoconvexo; ascis clavatis, $100-120~\mu \times 12-15~\mu$; sporiis biseriatis, ellipsoidis, $7-8\times 20~\mu$, 2-4 cellularibus; paraphysibus filiformibus, sursum clavulatis.

On bark of *Cornus* sp., East Hampton, New York, July, 1936. This is associated with a *Myxosporium* which appears to be its conidial stage. The spores ooze out from the pycnidia in sausage like streams, whitish in color. The conidia are ellipsoid, or slightly narrowed at one end, quite variable in size but often reaching a length of $40\,\mu$ and a diameter of $15\,\mu$, densely filled with minute granules.

EXPLANATION OF FIGURES

Fig. 1. Pezicula Corni. Center, photographs of several twigs showing apothecia; above, sketch of two apothecia, much enlarged; background, drawings of an ascus with spores; also two spores removed and the end of a ruptured ascus; below, drawing of a sorus and two conidia.

Fig. 2. Pezicula cornicola. Photograph of two branches showing apothecia. Above, sketch of apothecium, much enlarged; center, an ascus with spores; above right, two ascospores; below, a sorus with exuding conidiospores, much enlarged; below right, two conidia.

STUDIES IN THE GENUS MYCENA. IV 1

ALEXANDER H. SMITH

(WITH 3 FIGURES)

In the following account four species and one variety are described as new. One new combination is proposed and nine species are redescribed from studies of type material and fresh specimens. The writer is very grateful to Dr. Robert Kühner of Paris for his generous exchange of material, and his comments concerning many of the puzzling European species.

The collection numbers and photographs are those of the writer unless otherwise stated, and the specimens have been deposited in the Herbarium of the University of Michigan. The iodine solution used in determining the iodine reaction of the spores is made of five parts chloral hydrate, two parts water and an excess of iodine.

Mycena fuscoocula sp. nov. (Fig. 1, a, b, and c).

Gregaria; pileus 5–15 mm. latus, obtuse conicus demum campanulatus, glaber, fuscooculatus, demum pallide avellaneus; lamellae anguste adnatae, angustae, confertae vel subdistantes, sordide albidae; stipes 4–7 cm. longus, 1 mm. crassus, subviscidus, fuscus vel avellaneus, apice pallidus; sporae $10-12\times5-6~\mu$, ellipsoidae; basidia tetraspora; cystidia valde elongata, 65–95 \times 10–14 μ , levia. Specimen typicum in Herb. Mich. conservatum: legit prope Lake Quiniault, Wash., Oct. 19, 1925, C. H. Kauffman.

Pileus 5–15 mm. broad, subconic at first, becoming more or less campanulate in age, moist, glabrous, at first "fuscous" on the disk and "avellaneous" or "cartridge buff" elsewhere, in age drab to "pinkish buff" with a paler margin, margin somewhat sulcate striate, not viscid; lamellae ascending, narrowly adnate, narrow, close to subdistant, whitish; stipe 4–7 cm. long, filiform or up to 1 mm. dia. tubular, subviscid to the touch, concolorous with the pileus or paler above, often "avellaneous" except for the whitish apex, strigose; spores 10– 12×5 – 6μ , ellipsoid, bluish gray in iodine; cystidia very abundant on the sides of the lamellae,

¹ Papers of the Herbarium of the University of Michigan.

65–95 \times 10–14 μ , very conspicuous, those on the edge shorter and with the apex forked at times.

Gregarious on needles, Lake Quiniault, Wash., Oct. 19 and 20, 1925, collected by C. H. Kauffman. The above description is taken from Kauffman's notes. I have not seen fresh specimens. The species is amply distinguished by its stature which relates it

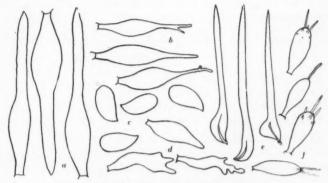


Fig. 1. a, b, c, Mycena fuscoocula; d, M. texensis; e, f, M. codoniceps aciculata.

to Mycena filipes (Fries) Quél., its long subcylindric pointed cystidia, and its large spores (four-spored basidia). Kauffman's notes describe the stem as subviscid and he had tentatively placed the species in the section Glutinipedes. In restudying the type, no gelatinous layer could be demonstrated. Consequently the species should be placed at least for the present in the old section Filipedes of Fries. Mycena Font-Queri Maire is apparently close to it, but is characterized by a shorter stem, thick walled cystidia and broad gills. The spores of the latter measure 10– 11×5 – 6μ , but the basidia are described as two-spored.

Mycena olivaceobrunnea sp. nov. (FIG. 3, c).

Gregaria; pileus 5–10 mm. latus, obtuse conicus, valde striatus, olivaceobrunneus demum sordide luteogriseus, glaber; lamellae subdistantes, adnatae, angustae vel sublatae, sordide luteogrisea, acie valde luteocitrinae; stipes 2–6 cm. longus, 1–1.5 mm. crassus, glaber, olivaceobrunneus demum pallide luteogriseus, tener; sporae 6.5–8 × 4–4.5 μ vel 8–10 × 4–5.5 μ , ellipsoideae; basidia tetraspora vel bispora; cheilocystidia fusoide ventricosa vel distorte ramosa.

Specimen typicum in Herb. Mich. conservatum: legit prope Lake Crescent, Wash., Sept. 20, 1935, A. H. Smith, n. 2507.

Pileus 5–10 mm. broad, obtusely conic, seldom expanding, moist, striate to the disk, "buffy brown" to "dark olive buff," paler on the margin, the striae dark and conspicuous, fading to sordid yellowish gray; flesh very thin and membranous, odor and taste not distinctive; lamellae subdistant, adnate, narrow or moderately broad at maturity, sordid yellowish gray, edge bright citron yellow; stipe 2–6 cm. \times 1–1.5 mm. polished, glabrous, "olive brown" below, "deep olive buff" or sordid yellowish gray above, rather weak and very fragile; spores 6.5–8 \times 4–4.5 μ or 8–10 \times 4–5.5 μ (two-spored), ellipsoid, pale bluish gray in iodine; basidia two- or four-spored; cystidia on the gill edge only, smooth, fusoid ventricose to saccate or with the apices more or less branched, occasionally with a few scattered blunt projections, 30–40 \times 8–18 μ ; pileus trama with a thin pellicle below which is a broad region of inflated cells, the remainder is filamentose.

Densely gregarious on humus and needles under second growth of Douglas fir, Lake Crescent, Wash., Sept. 20 (no. 2507-type), and Oct. 22, 1935 (no. 3279). This species is verly closely related to Mycena citrinomarginata Gillet, but the smaller spores, darker colors, and more delicate stature readily distinguish it. Mycena flavifolia Peck. is also very close but the spores of the type measure $7-8 \times 5-6 \,\mu$, and the cystidia on the gill edge are covered by numerous blunt projections. In addition, the gill edge is apparently not differently colored.

Mycena subsupina sp. nov.

Dense gregaria; pileus 3–7 mm. latus, conicus, centro fuscobrunneus vel olivaceobrunneus, demum avellaneus vel sordide albidus, sulcatus; lamellae distantes, angustae vel sublatae, adnatae; sordide albidae; stipes 1–2 (3) cm. longus, 1 mm. crassus, cartilagineus, fuscus vel cinereus; sporae (8) 9–11 \times 5.5–7 (7.5) μ , ellipsoidae; basidia bispora et tetraspora; cheilocystidia 36–50 \times 8–14 μ , levia, fusoide ventricosa, rare ramosa. Specimen typicum in Herb. Mich. conservatum: legit prope Oric, Calif., Dec. 5, 1935, A. H. Smith, n. 3782.

Pileus 3–7 mm. broad, narrowly to broadly conic, with a delicate bloom at first, soon polished, near "mummy brown" when young, disk sometimes darker, at maturity "buffy brown" or with an ochraceous tint, margin pale avellaneous to whitish and somewhat sulcate in age, fading to pallid gray or watery grayish brown, hardly hygrophanous; flesh thin, rather tough, odor and taste mild;

lamellae distant, narrow to moderately broad, adnate or slightly toothed, white to pale grayish, edge whitish; stipe 1–2 (3) cm. \times 1 mm. equal, rather tough and cartilaginous, concolorous with the pileus or paler, with a rather large cavity, glabrous and polished except for the white strigose base, base not rooting; spores (8) 9–11 \times 5.5–7 (7.5) μ , smooth, pale bluish gray in iodine, broadly ellipsoid; basidia two- or four-spored; cheilocystidia 36–50 \times 8–14 μ , smooth, fusoid ventricose with subobtuse apices, with the apex forked or with two to several finger-like prolongations above an inflated basal portion; pileus trama with a thin adnate pellicle, beneath it a region of slightly inflated cells, the remainder homogeneous.

More or less decumbent and densely gregarious on redwood logs, Oric, Calif., Dec. 5, 1935 (no. 3782-type). This species has the consistency and stature of Mycena supina, but the cystidia separate it at once. The spores, in addition, are characteristically ellipsoid rather than globose. Those of Mycena supina are typically globose to subglobose but vary to broadly ellipsoid in two-spored forms. Spores of M. subsupina are typically ellipsoid, but may be subglobose in two-spored forms.

Mycena texensis sp. nov. (FIG. 1, d).

Dense caespitosa; pileus 8–15 mm. latus, convexus demum late umbonatus vel subumbilicatus, viscidus, caeruleofuscus vel luteofuscus, demum cinereus vel luteocinereus; lamellae arcuate decurrentes, latae, subdistantes, pallide luteae, acie aurantiae; stipes 4–8 cm. longus, 1–1.5 mm. crassus, viscidus, apice pruinosus, luteogriseus vel aurantiogriseus; sporae 4.5–6 × 3–3.5 μ , ovoidae; basidia bispora; cheilocystidia 22–32 × 5–9 μ , fusoide ventricosa vel distorte ramosa. Specimen typicum in Herb. Mich. conservatum; legit prope Cisco, Texas, Sept. 8, 1935, E. A. Smith.

Pileus 8–15 mm. broad, oval to convex becoming broadly umbonate, the disk slightly depressed in age, glabrous, striate, margin subsulcate in age, viscid, nearly white when young, disk becoming bluish fuscous or dark grayish brown tinged with orange, the margin pale cinereous to whitish or tinged with orange yellow; flesh thin and pliant, odor and taste not known; lamellae moderately broad, arcuate decurrent, subdistant, whitish or pale yellowish to orange, margin deep orange (similar to Mycena Leaiana [Berk.] Sacc.); stipe 4–8 cm. × 1–1.5 mm., equal, viscid, covered by a dense orange pruinose covering toward the apex, the base densely white or grayish strigose and rooting somewhat in the substratum, color yellowish to orange above, becoming whitish, sordid grayish

or sordid brownish toward the base; spores $4.5\text{--}6 \times 3\text{--}3.5\,\mu$, bluish gray in iodine, ovoid; basidia two-spored (occasionally four-spored), $18\text{--}22 \times 4\text{--}5.5\,\mu$; cheilocystidia $22\text{--}32 \times 5\text{--}9\,\mu$, fusoid ventricose at first, soon with the apex more or less lobed or divided, sometimes with obtuse knob-like protuberances or variously contorted and irregular in outline; pileus trama with a thick gelatinous surface pellicle, more or less floccose below to the gelatinous subhymenium; gill trama with a gelatinous subhymenium when revived in KOH; stipe with a thick gelatinous layer over the surface.

Densely cespitose on oak logs and stumps west of Cisco, Texas, Sept. 8, 1935, collected by E. A. Smith. This species is closely related to *Mycena Leaiana*. The gelatinous surface pellicle of the pileus and stipe, the orange pruinose stem and yellowish to orange gills with the brighter edges and the densely cespitose habit all clearly relate it to that species. It differs in the very small spores, short narrow basidia and the grayish colors of the cap as well as in the shape and distribution of the cystidia. The above description is based on notes sent to me by Mr. Smith. I have not seen fresh material. However, the microscopic characters are at once striking and distinctive, and its relationship to *M. Leaiana* is obvious. When treated with chloral hydrate-iodine all the pileus trama except the gelatinous surface layer turns dark reddish brown.

Mycena rugulosiceps (Kauff.) comb. nov. (= Collybia rugulosiceps Kauff, Papers Mich. Acad. Sci. Arts and Letters 5: 126. 1926). This species was collected on several occasions in Washington, Oregon and California during the season of 1925. It was found on logs and sticks of Vaccinium and Alnus as well as on the wood of various coniferous trees. It also occurs on debris and sometimes apparently on humus. As in many of the larger species of Mycena, the margin of the pileus may be incurved at first or at least conivent with the stipe. As in Mycena megaspora Kauff. the gills occasionally stain reddish in age, particularly if the weather continues warm and wet. The colors of the pilei are variable, buttons are "blackish mouse gray" and as the cap expands the colors change to "drab" and finally become "avellaneous." The disk often remains a darker sordid brown. The cystidia measure 24–36 \times 8–12 μ , are clavate and the inflated portion is covered by rod-like projections. They are present only on the

gill edges. The spores measure $8-10 \times 6-7 \mu$, are broadly ellipsoid, and turn bluish gray in iodine. The pileus trama is characterized by a thin pellicle, beneath it is a compact region of enlarged pseudoparenchymatous cells (in tangential section), and the remainder is floccose and filamentose. The basidia are four-spored. This species is closely related to Mycena Berkleyi Massee, Mycena megaspora, Mycena Grantii Murrill, Mycena magna Murrill and Mycena longipes Murrill. From M. Berkleyi it should be readily separated by its larger spores. Mycena Grantii, which has no differentiated cystidia on the sides or edges of the gills and spores $5-6 \times 4-5 \mu$ (four-spored), is very close to M. Berkleyi. Mycena magna is characterized by spores $7-8.5 \times 6-7.5 \mu$ on two-spored basidia and by clavate-echinulate cystidia on the sides and edges of the gills $(28-34 \times 8-12 \mu)$. It should be readily distinguished from those mentioned above in either the two- or four-spored form. M. longipes is characterized by spores $8-10.5 \times 6-7 \mu$, and a very pale slender stem. Its cystidia are clavate to saccate (27-34 × $8-12 \mu$) and in the type specimen both smooth and echinulate individuals were found. The basidia are two-spored. M. longipes should differ from M. rugulosiceps in having smaller spores in the four-spored form as well as in its very pale slender stipe.

Mycena codoniceps (Cooke) Sacc. sensu Kuhner (4).

Pileus 1–3 mm. broad, obtusely conic, margin flaring in age, pale gray or brownish gray becoming sordid pallid gray in age, sulcate, at first densely setose, sparsely setose or glabrous in age, margin entire; flesh very delicate and fragile; lamellae narrow, narrowly attached or nearly free, subdistant, edge concolorous with the sides; stipe 1–3 cm. long, filiform, very soft and delicate, with a small rounded bulb at the base, gray, whitish in age, covered by setae, bulb also setose; spores $6-8\times3-4~\mu$, ellipsoid, pale yellowish or nearly hyaline in iodine; basidia two- and four-spored; cystidia on the gill edge only, $25-30\times7-9~\mu$, smooth, fusoid ventricose with blunt apices; pileus trama (revived in KOH) characterized by a narrow gelatinous pellicle above a vesiculose tramal body, numerous setae $150-200\times8-14~\mu$ arising in the pellicle and projecting, setae thick-walled, staining yellowish in iodine; stipe and bulb covered by similar or more elongated flexuous setae.

Scattered on spruce needles and leaves of Labrador tea, Rock River, Mich., June 17, 1933 (no. 33–537), and under clumps of ferns, Warrensburg, N. Y., Sept. 5, 1934 (no. 702). This is a characteristically gray species with large prominent setae, a gelatinous pellicle (at least when revived in KOH) and smooth fusoid-ventricose cystidia.

Mycena codoniceps var. aciculata var. nov. (FIG. 1, e and f).

Gregaria; pileus 1–3 mm. latus, conicus, sulcatus, caeruleogriseus demum sordide albidus vel pallide cinereus, dense setosus, tener; lamellae confertae, latae, anguste adnatae, sordide albidae vel griseae; stipes 1–3 cm. latus, 0.3–0.4 mm. crassus, griseus demum albidus, dense setosus; sporae 7–8 \times 2.5–3.5 μ , vel 8–10 \times 4 μ ; basidia tetraspora vel bispora; cheilocystidia clavata, sparsim aculeata. Specimen typicum in Herb. Mich. conservatum: legit prope Oric, Calif., Dec. 2, 1935, A. H. Smith, n. 3704.

The variety differs from the species in the bluish gray colors of the pileus which soon fade to sordid whitish, the broader closer gills which at times separate from the base of the stipe but, by adhering to each other, form an inconspicuous collar around it, and by the cystidia on the gill edge which have sharp aciculate more or less elongated projections scattered over their apices. The spores of both are yellowish in iodine. In water mounts of fresh material of both the species and the variety, the pellicle does not appear to gelatinize, but when material is revived in two per cent KOH the pellicle often swells up to 15-60 \u03c4 thick and appears very gelatinous. The variety has been found on redwood cones, bark and twigs, Oric, Calif., Dec. 2 (no. 3704-type) and Dec. 5, 1935 (no. 3783); on alder leaves, Yellow Dog River, Marquette, Mich., Sept. 9, 1933 (no. 33–912), and on debris under pine, Warrensburg, N. Y., Sept. 6, 1934 (no. 714). The fruit-bodies are not uncommon but are very easily overlooked and very difficult to collect because of the size and delicate consistency.

Mycena brevipes Murrill (fig. 3, b_1).

Pileus (5) 10–25 mm. broad, ovoid, becoming obtusely conic, conic campanulate or convex, at first hoary, soon polished, glabrous, surface moist, striate almost to the disk, "deep neutral gray" and fading to "pale neutral gray" on the disk, sometimes "pale drab gray" over all, the margin usually "pallid neutral gray" and

radially rugulose; flesh thin, fragile, pallid gray, not changing, odor and taste not distinctive; lamellae adnate, narrow to moderately broad, subdistant to distant at maturity, intervenose at times, whitish to pale cinereous, edge concolorous with the sides and even; stipe 1.5–3.5 cm. \times 1.5–3 mm., equal or the base subbulbous, concolorous with the pileus, pale cinereous or nearly white, at first covered by a powdery bloom, soon polished, the base surrounded by a mass of white strigose filaments or echinulate with white hairs radiating from the point of attachment, not rooting, occasionally the base stained sordid purplish brown in age; spores (7) 8–10 \times 5–6 μ , ellipsoid, bluish gray in iodine; cheilocystidia (30) 40–60 \times 10–18 μ , fusoid ventricose with obtuse apices or abruptly ventricose with a long aciculate protruding neck, smooth; basidia four-spored; pileus trama with a thin pellicle, beneath it a region of large inflated cells, the remainder filamentose.

Singly on the dead branches and twigs of coniferous trees or on the branches and twigs covering the forest floor as well as on decaying sticks of alder. Lake Crescent, Sept. 20 (no. 2512), Crescent Beach, Sept. 22 (no. 2550), Quillayute River, near La Push, Oct. 26 (no. 3351), and by C. H. Kauffman at Lake Quiniault, Wash., Nov. 4, 1925. In Oregon it was found at Lake Tahkenitch, Nov. 17 (no. 3514), Nov. 18 (no. 3532) and Nov. 25 (no. 3612); in California, at Trinidad, Nov. 29 (no. 3643), Oric, Dec. 3 (no. 3742) and Dec. 4, 1935 (no. 3755). The cystidia of the type are similar to those described above, but reliable spores could not be obtained. Murrill described the species as growing on hardwood sticks. Kauffman's collection was on Alnus, but all of mine were on conifers. The outstanding positive characters of M. brevipes are the short stem, top heavy appearance, habit of growing singly, pale gray color, fragile consistency, fusoid cystidia on the gill edge and lack of an odor or taste. It is very difficult to determine whether or not this fungus is merely a form of a previously described species from Europe. It is close to Mycena leptocephala (Fries) Gillet.

MYCENA ELEGANTULA Peck.

Pileus 1-2.5 cm. broad, remaining obtusely conic, glabrous, moist, at first "warm blackish brown" to "dark vinaceous brown" on the disk and "livid brown" to "brownish vinaceous" on the margin, at times "hellebor red" or with a fuscous tinge on the

disk and "pale rhodonite pink" near the margin, hygrophanous, fading but retaining a decided pinkish gray or vinaceous cast, sulcate striate in age; lamellae bluntly adnate, narrow, distant to subdistant, white, the margins pale or bright pinkish brown; stipe 3–8 cm. \times (1) 2–3 mm., equal, cartilaginous and brittle, glabrous except for the white strigose base, translucent above at times, grayish with a vinaceous tint at very first, becoming nearly "pale hydrangea pink" or brighter toward the apex in age, sometimes fading and becoming nearly white; spores 8–10 \times 5–6 μ , broadly ellipsoid, bluish gray in iodine; basidia four-spored; cystidia scattered on the gill edges, absent or rare on the sides, 30–40 \times 8–15 (20) μ , fusoid ventricose with obtuse apices, smooth, sometimes forking or branching at the apex, filled with a pinkish sap; pileus trama with a thin pellicle, below it a region of inflated cells, the remainder homogeneous.

Cespitose to scattered on conifer logs and debris, Lake Crescent, Wash., Sept. 22 (no. 2545), Oct. 19 (no. 3262), and Oct. 22 (no. 3295); Lake Tahkenitch, Ore., Nov. 18 (no. 3528) and Nov. 22 (no. 3583), and Florence, Ore., Nov. 22, 1935 (no. 3606). This species differs from Mycena purpureofusca, which is also common along our Pacific Coast, in its predominantly pinkish to vinaceous cast contrasted to the dominant lilac-fuscous colors of the latter. In addition the gill edge of M. purpureofusca is much darker. In consistency M. elegantula resembles Mycena inclinata (Fries) Quél. The smaller spores distinguish it readily from Mycena rubromarginata (Fries) Quél. The fungus described by Smith (7) as Mycena rubromarginata var. Laricis is the two-spored form of Mycena elegantula.

Mycena laevigata (Fries ex Lasch.) Quél.

Pileus 1–2 cm. broad, conic to convex or with a low subconic umbo, remaining broadly conic or convex, often with a small papilla, glabrous, lubricous or subviscid in age or when wet, at first pale fuscous watery gray or bluish gray on the disk and whitish toward the margin, soon fading and whitish over all, or at maturity with a cream colored disk, closely striatulate to near the disk, opaque at first; flesh thin, flaccid and cartilaginous, white, odor and taste not distinctive; lamellae moderately close and broad, broadly adnate-subdecurrent, white, edge even; stipe 2–5 cm. × 1–2 mm. equal, cartilaginous and tough, base white strigose and rooting somewhat in the rotten wood, glabrous, cartilaginous and

brittle, tubular, when young bluish gray above, pallid below, soon fading to watery grayish white or shining white; spores 6–8 \times 3–4 μ , broadly ellipsoid, bluish gray in iodine; basidia four-spored; cystidia basidia-like or elongated and with wavy outlines, 28–45 \times 6–11 μ , smooth, on the gill edge only; pileus trama with an upper region of compact pseudoparenchymatous cells (tangential section), the walls of the uppermost cells gelatinizing somewhat causing the slippery or subviscid surface, tramal body floccose filamentose.

Cespitose to subcespitose on conifer logs, North Fork of the Mad River, California, Dec. 9, 1935 (no. 3919), and on conifer logs, Bear Island, Lake Temagami, Ont., Sept. 9, 1936 (no. 4733). The spores in both of the American collections are slightly below the range in size usually given for the species in Europe. The lubricous pale pileus, cespitose habit, broadly adnate gills and smooth cystidia amply characterize it, however. The margin may be either connivent or slightly incurved. This in addition to the structure of the pileus relates it to lignicolous species of Collybia. Mycena radicatella Peck is a very closely related species usually found on the wood of deciduous trees.

MYCENA NIVEIPES Murrill (FIG. 3, a).

Pileus (1.5) 2-7 cm. broad, ellipsoid when young, convex to obtusely conic or becoming nearly plane, the margin slightly recurved, "clove brown" to "olive brown" or sordid drab when moist, striate, hygrophanous, fading to whitish or various shades of sordid gravish brown on the disk, margin white and sometimes sulcate in age, often splitting; flesh thin and very fragile, taste acidulous to subfarinaceous, odor nitrous or lacking entirely; lamellae broad, close to subdistant, narrowly adnate or slightly toothed, at first faintly bluish gray, soon fading to white, at times white from the first, in age occasionally flushed with pink, edge even or slightly fimbriate; stipe 4-10 cm. × 2.5-7 mm., very fragile, equal, hollow, pale bluish cinereous when young, becoming sordid or shining white, at first densely covered by a white fibrillose coating, longitudinally fibrous-striate in age or becoming glabrous, apex minutely scabrous at first, base more or less white strigose and sometimes subradicating; spores (7) 8–10 \times 5–6 μ , subglobose to ellipsoid, faintly bluish gray in iodine; cystidia abundant on the sides and edges of the lamellae, $50-90 \times 8-15 \mu$, smooth, narrowly fusoid, with subacute to acute apices, those on the edge often

fusoid ventricose and shorter (40–60 μ long); pileus with a well developed pellicle, beneath it a region of slightly inflated hyphal cells which grades imperceptibly into the typical floccose filamentose tissue below.

Singly to gregarious or subcespitose on old logs of oak, elm, ash, maple, etc., usually in the spring or early summer. The above description was drawn from collection no. 3967, Dexter, Mich., June 4, 1936. This is a common species in northeastern United States and eastern Canada. It is described as Mycena polygramma var. albida by Kauffman (3), who placed it in the latter species because of its frequently striate stipe. After collecting this species frequently during the past eight years, it is clear to me that Murrill (6) described one extreme variation as Mycena neveipes and Kauffman another. The spores of Murrill's type measure 8-10 \times 5-7 μ , and are broadly ellipsoid, the cystidia are abundant on the sides of the gills and measure $60-90 \times 8-12 \mu$. They are fusoid, smooth, and have subacute apices. The cystidia on the gill edge are $38-50 \times 10-14 \,\mu$, ventricose and smooth. The basidia are four-spored. Kauffman studied the species in its robust form as it develops in the spring. Murrill collected the late season form which is not as luxuriant and in which the fibrils on the stem are not conspicuous or soon disappear entirely leaving a shining white glabrous stipe. The odor is more apt to be absent in the late season form, but I have found collections of robust, odorless individuals in June. No. 971 from Warrensburg, N. Y., Sept. 2, 1934, and nos. 32-365, Stockbridge, Mich., Sept. 5, 1932, are the latest seasonal records I have of it. It is represented by no. 15195 in the Atkinson Herbarium at Cornell University, Ithaca, N. Y. (collected by H. S. Jackson), July 10, 1903. Atkinson had tentatively considered it as a new species. The large prominent cystidia, pale color, fragile consistency, and usually the odor separate it from M. polygramma (Fries) Quél. Mycena subalpina v. Höh. is apparently closely related to M. niveipes, but from the descriptions it is difficult to decide whether M. Jacobi Maire (M. pseudogalericulata Lange) is distinct from von Höhnel's species. The spores of M. Jacobi are apparently larger than those of M. niveipes.

MYCENA PECTINATA Murrill.

Pileus (5) 10-20 (30) mm. broad, obtusely conic with a flaring margin or subexpanded in age, when young covered by a distinct glaucous hoary coating, soon polished, glabrous, "benzo brown" to "fuscous" on the disk, fading through drab to pale gray with a whitish margin, the margin at first darker gravish brown and usually tinged vinaceous on buttons, striate to near the apex, sulcate in age; flesh very thin and papery in mature caps; odor and taste not distinctive; lamellae adnate, close to subdistant, narrow to moderately broad, white, sometimes yellowish in age, intervenose, edge whitish and even; stipe 1-4 cm. × 1-2 mm. "hair brown" and covered by a hoary coating, paler above, glabrous, base slightly enlarged at times and strigose with white hairs, very fragile; spores (7) $8-10 \times 4-5.5 \mu$, broadly ellipsoid, pale bluish gray in iodine; basidia four-spored; cystidia rare or scattered on the sides of the gills, more numerous on the edge, $40-50 \times 10-20 \mu$, with one or several finger-like prolongations or the apex mucronate, occasionally broadly fusoid ventricose; pileus trama with a thin pellicle, below it a broad region of inflated cells, the remainder filamentose.

Scattered to gregarious on sticks of Vaccinium and Acer and on needle beds under spruce in bogs. Common in the spring and early summer. The fragile consistency, thin sulcate brownish pileus with the whitish margin and broad, often mucronate cystidia distinguish it as a species. Murrill describes it as cespitose and up to 3 cm. broad, nos. 32-558 and 32-559 from a bog near Ann Arbor, Oct. 11, 1932, had pilei measuring 1-3 cm. broad. The microscopic characters of the type are the same as those given above, and the dried specimens are practically identical. It is closely related to Mycena metata but the cystidia distinguish it at once. Mycena tenuicula Murrill has similar cystidia but is easily distinguished by its consistency, more distant gills and tendency to stain reddish when bruised or in age. Mycena intertexta also has similar cystidia. I have not seen fresh material of either M. intertexta or Mycena avellanea Murrill, but a study of dried specimens indicates that they are very close to each other if not identical. The spores of the type of the latter measure $8-9.5 \times 6-7 \mu$. Both of these should be readily distinguished from M. pectinata by their densely cespitose manner of growth on decaying wood of conifers.

MYCENA POLYGRAMMA (Fries ex Bull.) Quél. (FIG. 2, a).

Pileus (1.5) 2-4 cm. broad, obtusely conic to oval at first, campanulate to plane or with an obtuse umbo in age, surface white canescent at first, the bloom often persisting until near maturity, glabrous and lubricous in age, color "fuscous black" beneath the bloom or "fuscous," fading slowly to "drab" or paler gravish. nearly "pinkish buff" at times, margin opaque and frequently sulcate, the surface often more or less uneven and appearing streaked with glistening lines, not hygrophanous; flesh very hard and cartilaginous, watery gravish to white, rather thin, odor none, taste mild; lamellae close, becoming subdistant as the pileus expands, broad (anteriorly), narrowed toward the stipe, narrowly adnate or with a short decurrent tooth, white or whitish, in age flushed with pink, often staining sordid brownish in spots, edge even; stipe 6-15 cm. long, 2-5 mm. dia. very brittle and cartilaginous equal, tubular, with or without a well developed pseudorhiza, base white strigose and often staining reddish brown, densely silvery longitudinally striate, twisted striate in some, "fuscous" or paler gravish brown beneath the silvery covering, at times nearly glabrous and smooth or glabrous and longitudinally grooved. apex pallid and faintly powdered, attached to sticks which are either on the surface or buried in the ground; pileus trama with a thick nongelatinous pellicle, beneath this a region of inflated irregularly arranged hyphae, the remainder of the floccose filamentose type; cystidia on the gill edge only, $26-34 \times (5)$ 7–10 μ , aciculate or the midportion somewhat enlarged and the apex forked or branched giving rise to two or several contorted fingerlike projections, spores $7.5-10 \times 5-6 \mu$, broadly ellipsoid, pale bluish in iodine; basidia four-spored.

Gregarious to subcespitose under maple and basswood, South Lyons, Mich., Oct. 6, 1936 (no. 5036). There has been much confusion in North America in regard to this species. The material cited above is similar in every respect to material from France by Dr. R. Kühner, and from England by Mr. A. A. Pearson. No. 16464 in the Atkinson Herbarium at Cornell University was collected by Atkinson near Paris and the determination checked by N. Patouillard. It and material sent to Atkinson by Romell are the same as the material cited above. Atkinson had also collected the species in New York (no. 18684). Tall slender forms of this species resemble Mycena pullata (Berk. & Cooke) Sacc. or Mycena praelonga Peck in appearance. The former is

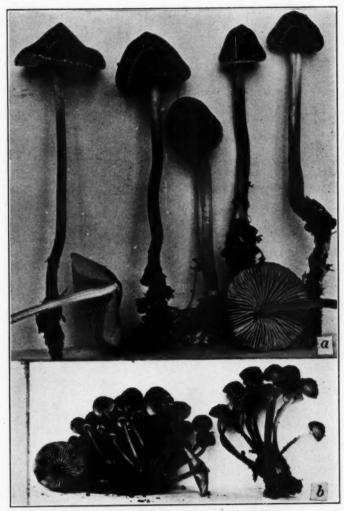


Fig. 2. a, M. polygramma; b, M. tintinnabulum.

readily distinguished by its color and the latter by its relationship to M, alcalina and habitat on sphagnum.

Mycena tintinnabulum (Fries) Quél. (Fig. 2, b). (= Omphalia semivestipes Peck, Bull. Torrey Club 22: 200. 1895.
Omphalia curvipes Peck, Bull. Torrey Club 34: 345. 1907.
Mycena subalcalina Atk. Am. Jour. Bot. 5: 37. 1918.)

Pileus 1-3.5 cm. broad, convex to plane or slightly umbonate, blackish or with a bluish black sheen when young, slowly fading and becoming grayish or sordid whitish in age, at times the margin becoming ochraceous tinged, in wet weather often staining sordid reddish brown, lubricous to subviscid, opaque when moist, becoming closely translucent striatulate on the margin when fading, margin straight or slightly incurved at first; lamellae narrow to moderately broad (2-3 mm.), close (subdistant in broadly expanded pilei), broadly adnate or slightly decurrent, pallid to white, at times pallid fuscous in age, often tinged sordid flesh color or with reddish brown spots, edge even and whitish to grayish; stipe 2-6 cm. \times 1-3 mm. tubular, terete or compressed above, slightly pruinose, soon polished, concolorous with the pileus or paler, becoming reddish or blackish brown at the base in age, base white strigose, very tough and cartilaginous; spores 4-5 $\times 2-3 \mu$ (5-6 $\times 3 \mu$ or 3-4 $\times 2 \mu$), pale bluish gray in iodine, smooth, broadly ellipsoid; cystidia rare or scattered on the sides, $25-30 \times 9-11 \,\mu$, saccate, occasionally with knob-like processes at the apex or smooth and with wavy outlines, those on the gill edge similar or scarcely differentiated from sterile basidia; basidia fourspored; pileus trama with a thin gelatinizing pellicle, beneath it a region of slightly enlarged cells, the remainder floccose filamentose, becoming reddish violet in iodine; odor strongly alkaline at first but soon vanishing, frequently entirely absent.

Densely cespitose or densely gregarious on old stumps and logs, particularly of basswood. This is a common species during wet cool weather in late October or November throughout the Great Lakes Region and eastern North America. Overholts (no. 1452; 1526; 2315) has collected it in Missouri and Ohio, and Hesler has recently found it in Tennessee during the month of January. The microscopic characters of the type of *M. subalcalina* and *O. curvipes* are the same as those given in the above description. Expanded fruit-bodies in my collections correspond very closely to the illustration of Fries (2), and his description (1) of the macro-

scopic characters covers my collections very well. Material so determined in the Atkinson Herbarium at Cornell University, which was sent to Prof. Atkinson by Romell, has the characteristically small spores and the thin gelatinous pellicle over the

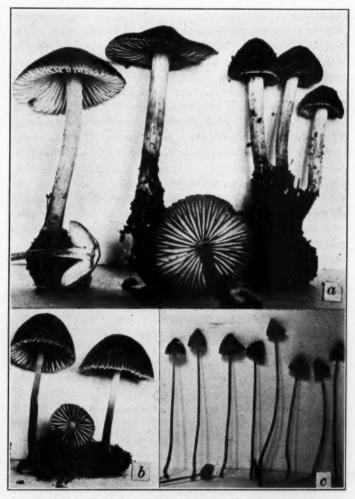


Fig. 3. a, M. niveipes; b, M. brevipes; c, M. olivaceobrunnea.

pileus. Meier (5) points out that the spores as given by Ricken are too large and states that in his own collections they measure 5.5-2.7 µ. The broadly adnate-subdecurrent gills led Peck, who had collected the small odorless form, to place it in Omphalia. Atkinson placed great emphasis on the odor as is indicated by the name he gave it. My experience with this species shows that the odor is a very unreliable character. It is often very strong while the fruiting bodies are attached to the substratum, and I have located clumps twenty to thirty feet away by "following my nose." However, within a few minutes after collecting these specimens the odor was no longer present and was not detected at any later time during the process of studying and drying them. On other occasions I have collected perfectly fresh material in which the odor was not present. The type of Omphalia semivestipes is very much like a large fruit-body of M. tintinnabulum. Its spores measure $5-6 \times 2-3 \mu$, and a thin gelatinous pellicle covers the pileus. No differentiated cystidia were found, but since they are nearly always rare and not greatly enlarged, this difference is not taxonomically important. The strigose covering over the lower portion of the stipe should not be considered important either since it is obviously the portion which was buried in the substratum. Most of the lignicolous species of Mycena in the old section Rigidipedes of Fries are characterized by a subradicating strigose stem base. The most reliable characters of M. tintinnabulum are the small spores, broadly adnate-subdecurrent gills, poorly differentiated cystidia, pliant cartilaginous consistency and lubricous to subviscid pileus.

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MYCOLOGICAL NOTES. I

C. L. SHEAR

Under the above title the writer proposes to present a series of notes on various genera and species of fungi. These records have been accumulating for many years. They are based chiefly upon the examination of type or authentic specimens of the species of the older mycologists. The brief and imperfect descriptions of the early authors have led to many doubtful or erroneous determinations of their species. The writer has fortunately had an opportunity during the past 40 years to examine the herbaria of most of the older European and American mycologists for which he wishes to express again to those of the curators and directors of the various herbaria who are still living his appreciation and gratitude for the kindnesses and facilities provided.

1. Discella effusa B. & B. = Glomerella cingulata (Stone) S. & V. S.

CONIDIAL FORM

A specimen in Michener's Herbarium, labelled "Discella effusa B. & C.," on a partly, decayed apple collected by Michener in Pennsylvania, No. 647, is evidently part of the type collection. The last "B" in the authority as printed is probably a typographical error for "C." If it were intended for Broome, it would probably have been written "Br."; the usual form of abbreviation used by Berkeley. The number 3541 given by Berkeley in his "Notices of North American Fungi," No. 466, Grevillea 2: 100, 1874, probably refers to the number under which Michener's specimen was sent to him. It appears that Curtis did not use Michener's numbers in transmitting his collections to Berkeley for identification. The specimen shows a typical circular decayed area of an apple in which the spore masses have nearly all been eaten away by insects or washed away, leaving only the acervuli surrounded by whitened portions of the epidermis of the fruit

and thus giving them a slightly superficial suggestion of *Discella*. The spores are typical of the bitter rot of apple. Spore measurements given by Berkeley are ".0008 long \times .00016 wide."

2. HERCOSPORA TILIAE Fries and RABENHORSTIA TILIAE Fries

The genus, *Hercospora* is frequently attributed to Tulasne. It was, however, published by Fries in his Syst. Orb. Veg. p. 119, 1825. Following the description he says: "The types are *Sph. Tiliae* and *Sph. atrovirens.*" The type as interpreted by Tulasne and later authors is *H. Tiliae* Fries. Fries did not actually write the combination *H. Tiliae* and it is usually attributed to Tulasne. In Sum. Veg. Scand. p. 397, 1849, Fries again describes the genus, but does not mention or include either of the species given in Syst. Orb. Veg. 1. c. This fungus is very fully described and illustrated by Tulasne, Sel. Fun. Carp. 2: 154–158, Pl. 18, figs. 1–18, and Pl. 19, figs. 1–14. The fungus is not given in Ellis & Everhart, N. A. Pyren., but it is mentioned as a possible synonym of *Melanconis Tiliae* Ellis, p. 525, and there are very few American specimens in the Bureau of Plant Industry herbarium. All are on *Tiliae*. They are as follows:

No. 2522—Ellis & Everhart, N. Am. Fungi, collected in Canada by Dearness.

No. 2952—Ellis & Everhart, N. Am. Fungi, collected at Wilmington, Del., by Commons.

No. 1421—Shear, 1903; also without number, Washington, 1910, Bliss, Iowa, 1927.

No. 1834—Nuttall, from W. Va.

No. 3990—Shear on old cut branches of Tilia sp., Roaring Gap, N. C., 1934.

Most of the specimens of this in the herbarium both European and American are poorly developed, showing only small stromata and the perithecia few or none. The species is well marked, however, in this condition by a distinct circular black line surrounding the light colored tissue of the bark in which the perithecia appear. The perithecia are frequently associated with *Rabenhorstia Tiliae* Fries, which is regarded by Tulasne, Winter and others as its pycnidial form, but this has not been proven by pure cultures so

far as we know. Wehmeyer apparently did not study the fungus, as we find no mention of it in his papers. The Roaring Gap specimens cited are fully developed in both the pycnidial and perithecial stages. The pycnidial form agrees with that described and illustrated by Tulasne, l. c. The pycnidia are obtuse, conical, thick, black and surmounted by a depressed globose mass of nearly hyaline pycnospores, $12-15 \times 6 \mu$. Tulasne says $11-13 \times 6\frac{1}{2} \mu$. The locules are located on the sides of the inner wall of the pycnidium and the base is poorly developed, and does not form a distinct wall. On this account the pycnidia are very easily detached from the bark and soon disappear after fruiting, leaving only a slight dark colored depression surrounded by the epidermis. The perithecia form from a stroma in the same spot left by the base of the pycnidium, and in our Roaring Gap specimens the perithecia have formed and the ostioles grown up about the old neck of the pycnidium. The ascospore measurements as given by Tulasne are $16-22 \times 10 \,\mu$. I have examined various European specimens, as follows:

Herb. Bresadola, Gocciadora, Apr., 1923, spores 15–19 \times 7–9 μ . Sacc. Myc. Ven. No. 676, spores 15–18 \times 7–8 μ .

Petrak No. 417, spores $13-18 \times 6-8 \mu$.

Krieger, Fun. Sax. No. 381, 15-18 \times 6-7 μ .

Winter gives ascospore measurements of this species as 24–26 \times 12–13 μ .

As will be noted the measurements of Tulasne and Winter exceed those of any European or American specimens we have seen. They would seem to be incorrect; or is there another species in Europe? The American specimens cited vary from 15– 19×7 – $9 \,\mu$. They are very variable in shape, and when fresh the spore contents show a rather peculiar vaculose granular appearance. They become 1-septate at maturity, but we have seen none showing color. Sometimes there is a slight constriction at the septum. It seems probable that the fungus is rather widely distributed in this country wherever the host occurs, but that it is not often found in a well developed condition.

Traverso says: Fl. Ital. Cryptogama 2: 189, 1906, that the ascospores are $18-24\times8-10\,\mu$ and cites Sacc. Myc. Ven. No. 676

as above, but we found none so large in our specimen of this number. He says *Hercospora* is distinguished from *Melanconis* by its different pycnidial form, and the pseudostroma which is limited by a black zone surrounding the perithecia.

3. Naumovia Dobr., 1928, Rosenscheldia Speg., 1883, Gibberidea Fuckel, 1869, and Melogramma Fries, 1849

NAUMOVIA Dobr., Bolez. Rast. (Morbi Plant.) 16: 197. 1928.

The type species, N. abundans Dobr., described and illustrated, 1. c., is represented by two specimens in the Bureau of Plant Industry Herbarium, one on dead stems of a Labiate, probably Prunella vulgaris collected by the writer above Chain Bridge, Va., May 31, 1920, and another on Prunella vulgaris collected at Hartford, Wash., July 1920, by C. R. Stillinger. The first specimen referred to is not quite mature, the spores only showing faint color in mass and obscurely septate; but agrees in every respect with Dobrozrakova's description and illustration, except that she says that there are no paraphyses present. Our specimens show clearly filiform paraphyses or rather what Petrak would call paraphysoids, being attached at both the base and apex of the perithecium or locule, forming apparently a sort of tissue-like network, though branches are not easily demonstrated. The structure is very similar to that found in the kernel of Physalospora and Botryosphaeria, the young perithecia being packed with a white tissue-like mass characteristic of the Pseudospheriaceae. The second specimen from Washington State is in a young condition and shows this character, the compact white center of the locules, very distinctly.

Comparing these specimens with Rosenscheldia Heliopsidis (Schw.) T. & S., the similarity in all the characters of the specimens both macroscopic and microscopic is very marked. About the only difference to be observed is in the size and shape of the spores which in the latter species are $28\text{--}30~\mu \times 4\text{--}5~\mu$, and in the former according to the author $30\text{--}39\frac{1}{2}\times 1\frac{1}{2}\text{--}3~\mu$. The author says 1--6 septate, though 4 are the most shown in his illustration and 3 is the usual number in our specimens.

A good mature specimen of Dobrozrakova's species on *Prunella vulgaris* from Lake Temigami, T. F. E., Ontario, No. 3509, was sent by Dr. Jackson, collected June 20, 1932; also another specimen on *Mentha* sp., from Morristown, Nova Scotia, collected by A. Rolland June 29, 1934. It is clear from a study of these specimens and the illustrations that *Naumovia* Dobr., 1928, is a synonym of *Rosenscheldia* Speg. 1883, differing only in its narrower ascospores which possibly become more septate.

Rosenscheldia Speg. Anal. Soc. Sci. Argent. 19: 250. 1886

The type of the genus Rosenscheldia is R. paraguaya Speg. l. c. The original specimens of this were examined and fully described by Theissen & Sydow, Dothidiales, 648–49, and illustrated with a diagrammatic section of a stroma with locules, Pl. 4, fig. 11. The separate, closely gregarious or caespitose, sub-stipitate locules are without true ostioles, but rupture at the apex when mature by dissolution (lysigeny) of the tissue. The asci are in a mass arising from the base; paraphyses (paraphysoids of Petrak) present; spores becoming 4-celled, fusiform, pointed, 27– $31 \times 3 \mu$, grayish brown, indistinctly septate.

Melogramma Fries 1849

Von Höhnel in discussing the above species, Frag. Myc. 13: No. 708, after studying Roumeguere's Fungi Sel. Exs. No. 4155, which Theissen & Sydow cite as Spegazzini's species, says that it is very similar to Melogramma vagans and that Spegazzini's type should be referred to Melogramma. We have studied a specimen of Gibberidea obducens Rick which Theissen & Sydow say is the same as Spegazzini's type R. paraguaya, and also Montagenella Heliopsodis (Schw.) Sacc., and of which we have abundant material, which Theissen & Sydow regard as a typical Rosenscheldia. These are evidently closely related species having the same internal structure of the locules and congeneric.

Theissen & Sydow, I. c., 649, say that as the type of Rosenscheldia is a genuine Dothideaceous fungus, to compare it with typical Sphaeriaceous perithecia (as in Melogramma) is out of the question. This remark refers to von Höhnel's opinion just noted in regard to its relation to Melogramma. A careful study,

however, of M. vagans, the type of Melogramma in different stages of development shows that there are no separate paraphyses, but an indistinct network of more or less filiform, gelatinous hyphae surrounding the kernel of the locule, which becomes modified toward the apex and appears as paraphyses just below the ostiole. The inner wall of the locule is distinctly darker colored than the surrounding stroma, but not readily separable from it. The ostiole is somewhat like that in the Sphaeriaceae but rather intermediate between that and Rosenscheldia. These genera are closely related, but seem generically distinct. Melogramma is evidently a connecting link between the Pseudosphaeriaceae and the Sphaeriaceae. There is a difference of opinion among students of these fungi in regard to the presence or absence and character of the paraphyses. Von Höhnel says that Melogramma has abundant paraphyses. Tulasne also describes and figures paraphyses showing a few irregular filiform hyphae. Winter and others say paraphyses are present; but, as has been noted above, we find no true paraphyses intermingled with the asci. Such more or less filiform and united hyphae as surround the kernel of asci do not readily separate and can scarcely be considered true paraphyses. Theissen & Sydow and von Höhnel describe Rosenscheldia as having abundant filiform paraphyses. As already stated the so-called paraphyses are only found as separate filaments of the network of hyphae in which the asci are developed and which tend to become separate at the maturity of the asci and finally disappear.

GIBBERIDEA Fuckel, Symb. Myc. 168. 1869

The monotype of this genus, G. Visci Fuckel, l. c., appears to be a rather rare fungus and confined to the host, Viscum album, so far as known at present. Petrak, Mycologische Notizen, Ann. Myc. 23: 58–61, 1925, reports a study of typical specimens of this species, his number 2099, Fl. Boh. & Mor. Exsic. He says this has the same structure of perithecia or locules and kernels as in the Cucurbitariaceae, and that it scarcely differs from Rosenscheldia as represented by R. Heliopsidis (Schw.) Theiss. & Syd. The structure of the kernel completely agrees, but the stroma is somewhat different and the genus (Gibberidea) may be kept

separate for the present on that basis. An examination, however, of Petrak's specimen No. 2099 shows that it agrees in structure with Rosenscheldia obducens Rick which is the same as R. paraguaya Speg., the type species, in practically every particular except that it has slightly less basal stroma and different sized spores. It also agrees in all essential characters with Naumovia Dobr., except for spore size. Dearness who examined a specimen of G. obducens from Rick which Weir sent him as No. 20,718 has a note with the specimen saying "I propose Pseudomeliola Menthae as n. sp. pro tem." The asci according to his measurements were $60-100 \times 6~\mu$, the spores $30-42 \times 2\frac{1}{2}-3\frac{1}{2}~\mu$. The specimen is typical G. obducens of Rick. Dearness adds that he has not seen the description of Rick's species. We have examined the following specimens of it:

No. 75 from the herbarium of Bresadola on living stems of *Mentha* sp. collected by Rick in Brasil. No. 1561 Rehm Ascom. Exs. on living stems of *Mentha*, São Lopoldo, Brasil, Rick. Spores 30–39 × 3. No. 20,718 Herb. Weir on *Mentha* collected by Rick, same locality as above; also another on a Labiate plant from São Cruz, Brasil, 1927, Rick.

All these specimens are typical *Rosenscheldia* and equal *R. para-quaya*, the type of Spegazzini's genus.

It is interesting in this connection to note that Dobrozrakova, Morbi Plant. 16: 212, 1928, says in comparing his Naumovia with the type of Gibberidea, G. Visci, that it differs generically in belonging to the Scolecosporae. He also compares G. obducens (Rosenscheldia paraguaya) with his species and concludes that it should be transferred to Naumovia. He was apparently not familiar with the genus Rosenscheldia, of which Gibberidea obducens was really the type.

From the above discussion it is concluded that Naumovia Dobr. 1928, Rosenscheldia, Speg., 1883, and Gibberidea Fuckel, 1869, are synonyms and that the following species should be called Gibberidea: G. Visci Fuckel, G. paraguaya (Speg.) n. comb., G. Heliopsidis (Schw.) n. comb., and G. abundans (Dobr.) n. comb.

4. PLEUROSTOMA Tul. 1863 and NEOARCANGELIA Berl. 1905

Recently abundant material of Sphaeria ootheca Berk. & Curt. Coronophora ootheca (B. & C.) Sacc. was found on dead oak near

362

Rosslyn, Va. We find by an examination of Schweinitz' specimen and as indicated by Berkeley, that it is S. mucida var. rostellata, Schw. Syn. N. Am. Fungi, No. 1515, 1832. Von Höhnel, in Myc. Frag., Ann. Myc. 16: 129 and 131, 1918, says that Neoarcangelia Berl. Icon. Fung. 3: 6, 1905, is identical with Pleurostoma, Tul. Sel. Fung. Carp. 2: 247, except that in the former the ostioles are more nearly vertical as shown in No. 2078, Rehm Asco. Exsic. A comparison of our specimens with the description and illustration of Berlese, l. c. pl. 7, which were drawn from the original American material described by Berkeley & Curtis, indicates that they agree in every particular, except that there is the greatest variability in the orientation of the perithecia which show all intermediate conditions between those with ostioles pointing laterally, to those exactly vertical. Apparently typical material of Pleurostoma Candollei Tul. 1. c. distributed by Weese in Eumycetes Sel. Exsic. No. 20 on oak, agrees in all particulars with the descriptions and illustrations of Tulasne and of Berlese, except that we do not find any such distinct substipitate base to the perithecia. Most of them are entirely sessile, lying rather loosely, but slightly attached to the substratum so that they are easily removed by a light touch. Comparison of asci and spores of Berkeley's specimens and ours indicate that the two genera are not only identical, but P. Candollei and N. ootheca are one and the same species. The asci, which are supposed to be somewhat different in shape are most variable, ranging from subglobose to pear-shape or clavate. The size of the spores covers the same range in both cases. According to Tulasne, the ascospores are 3.5μ long; according to Berlese they are $4-5 \times \frac{1}{3} \mu$ and in N. ootheca $2-3 \times 1$. We find in neither species ascospores exceeding 3.5μ in length.

As to variability in the direction of the ostioles, this appears to be simply the result of the physical conditions under which the perithecia develop. According to descriptions and to the material we have examined, the perithecia arise on the surface of the wood beneath the bark of dead and dying oak branches. The bark is apparently so thick and hard that the ostioles are unable to penetrate it; therefore they turn more or less to one side on account of the pressure from above.

The fungus is evidently closely related to *Romellia* Berl., differing only in the shape of the ostiole and the polysporous asci. It differs from *Coronophora* to which it was referred by Saccardo in having the asci arranged on branching stipes and the perithecia in valsoid groups. The fungus may not be rare in this country, as it is easily overlooked on account of the location of the perithecia, which appear to remain covered by the bark for a long time and show no external indication of their presence.

SPHAEROPSIS UVARUM Berk. & Curt. Grevillea 3: 1, September 1874

This species is represented in the Curtis collection in the Farlow herbarium by a specimen numbered "4031, Curtis" on a Scuppernong grape, collected, according to the label, September 1853, at Society Hill, South Carolina. This is the only number and only specimen cited by Berkeley in the original description. A careful comparison of sections of this specimen with the descriptions and figures of Viala, and Istvanffi in his monograph on Coniothyrium diplodiella (Speg.) Sacc. as well as with specimens of this species distributed by Briosi and Cavara in their Fun. Parasit. Plan. Colt., No. 48, collected at Como, Italy, September 1887, shows that the two organisms are the same. The external characters of the specimen in the Curtis collection and that of Briosi and Cavara as cited are identical. The appearance of this fungus under a hand fens, as indicated by these two specimens, is apparently characteristic and easily recognized. It seems to be distinguished from all the other fungi occurring upon the grape by the grayish, furfuraceous appearance of the pycnidia, which are very numerous and rather closely arranged, or sometimes confluent over the surface of the shriveled berry. This gravish furfuraceous appearance seems to be due to a sort of efflorescence of the epidermis of the grape, covering the pycnidia and thus obscuring the naturally dark color of their walls. All the macroscopic and microscopic characters of these two specimens agree so closely that there can scarcely be a doubt as to their identity. This is apparently a rare species in this country.

BUREAU OF PLANT INDUSTRY, WASHINGTON, D. C.

NOTES AND BRIEF ARTICLES

Professor Bohumil Shimek, a charter member of the Mycological Society, was born in Johnson County, Iowa, June 25, 1861 and died at Iowa City, January 30, 1937. Except for two years spent as an instructor at the University of Nebraska, the bulk of his life was spent in Johnson County. Graduated from the State University of Iowa as a civil engineer in 1883, his connection with the University as a teacher began in 1890 and continued until his death. Successively, instructor, assistant professor (1893), professor (1903) and head of the department of botany (1914) he retired from the latter position in 1919 to devote himself more freely to his studies. In 1932 he was made research professor. In 1913–14 he was exchange lecturer at the University of Prague, from which institution he received an honorary Ph.D.

Professor Shimek's interests in natural science were unusually varied. Trained as an engineer, he did active field work in geology and for a time taught zoölogy. Several of his papers, especially those dealing with the problem of the loess and its fossil contents, reflect these interests. He was an indefatigable collector and used to say that in the course of his work he had walked across every one of the ninety-nine counties of Iowa. In addition he took extensive trips to other regions, including Nicaragua, New Mexico and the Gulf states. He published little or nothing on the fungi, but collected assiduously, and the mycological collections of the University of Iowa contain many of his specimens. From his very last trip, to the Mississippi sand dunes, in November, 1936, he brought in several uncommon species.

His chief interest was in the ecology of the prairie and he became known as one of the best informed American botanists on this subject. He was an ardent and militant conservationist, maintained a vigorous interest in civic affairs and was active and influential in Bohemian societies in the United States.

In 1886 he married Anna Konvalinka of Iowa City. After her death he married Margaret Meerdink, who was his constant companion on his later collecting trips and who survived him only a little over two months.

Professor Shimek was almost the last of the old-fashioned naturalists—men who were interested in every phase of natural history and who insisted upon familiarity with their material under natural conditions. Without deprecating the intensive specialization and necessary laboratory restriction of much of our modern study, it may be maintained that the combination of broad background and extensive field work exemplified by naturalists of this type has made an important contribution to the general biological picture and may again prove a necessary approach to many of our more general problems.—G. W. MARTIN.

MYCOLOGICAL SOCIETY OF AMERICA

THE SUMMER FORAY, SEPTEMBER 3-5, 1936

(WITH 4 FIGURES)

Through the courtesy of Professor Ivey M. Lewis, director of the Mountain Lake Biological Station, the Society was able to hold its summer meeting at Mountain Lake, Virginia. This station, situated approximately 4000 feet above sea level furnished the members excellent laboratory facilities with plenty of working room, and at the same time comfortable sleeping quarters were available in the cabin type of dormitories, each of which was equipped with an open fireplace before which members gathered to talk and to bask in the cheer emanating from the wood fires.

As was true nearly everywhere else, the summer had been dry, but just prior to our arrival on September third, there had been a few small showers which furnished sufficient moisture to start the growth of fungi. In the beech woods mixed with maple and other broad-leaved trees of the region, and in the hemlock woods and Rhododendron thickets along the ravines below the camp, the covering of the forest floor was amply moist to support the growth of a wide variety of fungi. In the relatively open oak woods

around and above the camp, however, there was less moisture and fewer of the larger fungi. This state of affairs was soon remedied, for the day after the arrival of the main contingent, cool rains fell, followed by heavy mists, and it was not long before the agarics were pushing up on all sides. As a result, collecting was all that one could ask for, whether the main interest were in the smaller ascomycetes or in the larger basidiomycetes, as is witnessed



Fig. 1. Mountain Lake.

by the fact that 373 different species were collected during the foray.

A not to be forgotten occasion was that furnished by Mr. John B. Laing, a sympathetic and generous supporter of the Biological Station, who invited the members of the Mycological Society to a picnic on his property at the Cascades of the Little John River, a spot of wild beauty. The approach to this place was via a wood road that passed through woods of white pine or of the broad leaved species of trees and was therefore not unusual for that part of the country, but then suddenly on taking a sharp bend one

entered a beautiful stand of hemlocks, and looking down through this one could see through the lace-work pattern formed by the trees, the beautiful cascades of water falling over a cliff into the depth of a rocky gorge made on one side by the steeply sloping hill-



Fig. 2. Cascades of the Little John River. An idea of the height of the falls may be obtained by comparison with the people on the bridge in the foreground.

side covered with trees, and on the other by the almost vertical wall of stratified rock covered with a rich vegetation of bryophytes, ferns, and herbaceous plants that made a lush growth in the humid atmosphere created by the tumbling waters. In these surroundings, on a rock island in the middle of the stream and not far from the base of the falls, the members of the Society enjoyed an esthetic as well as gastronomic feast. But even in this setting, the collecting instinct came to the fore, and not long after lunch, members could be seen collecting along the less steep side of the gorge. It is certain that all of us who were privileged to visit this



Fig. 3. Looking eastward from Eagle Rock.

place of beauty, greatly appreciated Mr. Laing's hospitality and generosity.

The following day the party was divided in its interests. A portion decided to remain in the vicinity of the Station to carry on with their collecting, while the other part, after lunch, followed the trail above the camp to Eagle Rock and Bald Knob. The collecting on this trip was very good, but scarcely to be compared with the striking views that were obtained from several vantage points on the way to and at the summit of Bald Knob. Eventually the

trail led down to Mountain Lake which was bordered on its eastern shore by a small "forest" of Rhododendron and mountain laurel. This "forest" was well worth visiting and certainly worthy of more intensive collecting, but the thought that kept forcing itself on our minds was what a sight this place must be earlier in the year when the laurels were in full bloom. However, all who went on the trip felt well repaid by the specimens collected and the scenery they had enjoyed.



Fig. 4. Back row: Dr. G. S. Torrey, Mr. Andy Ingalls, Dr. Ivey F. Lewis. Middle row: Mr. Thomas Brown, Mr. Fulton, Miss Hagelstein, Mrs. Robert Hagelstein, Mrs. G. S. Torrey, Miss Overholts, Mrs. H. R. Fulton, Miss Fulton, Mrs. Walter H. Snell, Mrs. W. W. Diehl, Dr. H. R. Fulton, Mr. Thomas. Front Row: Mr. John A. Stevenson, Dr. L. O. Overholts, Dr. Walter H. Snell, Dr. C. L. Shear, Dr. D. H. Linder, Mr. Robert Hagelstein, Miss Edith Cash, Miss Anna E. Jenkins, Dr. W. W. Diehl.

It was the general consensus of opinion that the foray was decidedly well worth while, since not only did it give the members an excuse to get away from their usual haunts, but also furnished them an opportunity to gather in an informal way and be sociable with kindred souls. For the pleasure and success of the foray, a debt of gratitude is owed to Dr. and Mrs. Ivey Lewis whose genial hospitality and whose keen interest and coöperation made it possible to do so much in so little time. Also many thanks are due to Miss Proffitt who so ably assisted Dr. Lewis, and to Mrs. Feil who saw to it that the members were well fed, and finally Messrs. Andy L. Ingalls, Thomas D. Brown, Walton C. Gregory, and Charles Maphis of the laboratory staff deserve our sincere thanks for their ever willing helpfulness and for guiding us over the various trails that led through the excellent and varied collecting grounds.

The following attended the foray (non-members preceded by an asterisk): Miss Edith Cash, Dr. and *Mrs. W. W. Diehl, *Mr. and *Mrs. Walter H. Snell, Mr. John A. Stevenson, *Mr. Thomas, stein, *Miss Hagelstein, Miss Anna E. Jenkins, Dr. D. H. Linder, Dr. L. O. Overholts and *Miss Overholts, Dr. C. L. Shear, Dr. and *Mrs. Walter H. Snell, Mr. John A. Stevenson, Mr. Thomas, and Dr. and *Mrs. G. S. Torrey.

In making up the list of species collected at Mountain Lake, the writer has, for the sake of brevity, omitted the names of collectors and in their stead has indicated by symbols the herbaria in which the specimens have been deposited. Thus, if anyone should desire to write up a flora of the region involved or of the state of Virginia as a whole, these specimens might be more readily located and additional data obtained from them. The symbols, represented by letters in parenthesis, are as follows:

- (F) = Farlow Herbarium, Harvard University.
- (H) = Herbarium of Robert Hagelstein or of the New York Botanical Garden.
- (O) = Overholts Herbarium or that of Pennsylvania State College.
- (S) = Herbarium of Walter H. Snell or of Brown University.
- (W) = Bureau of Plant Industry, U. S. Department of Agriculture, Washington, D. C.

MYXOMYCETES: Arcyria cinerea (Bull.) Pers., (H); A. denudata (L.) Wett., (H); A. digitata (S.) Rost., (F); A. globosa Schw., (F, H, W); Badhamia rubiginosa (Chev.) Rost., (H); Ceratiomyxa fruticulosa (Muell.) Macbr., (H); Comatricha typhoides (Bull.) Rost., (H); Cribraria purpurea

Schrad., (H); Diachea leucopodia (Bull.) Rost., (H); Diderma floriforme (Bull.) Pers., (F); Diderma testaceum (Schrad.) Pers., (F, H, W); Didymium nigripes (Lk.) Fr., (F, H); Enteridium Rozeanum (Rost.) Wing., (F, W); Hemitrichia serpula (Scop.) Rost., (F, H); H. stipitata (Mass.) Macbr., (H); H. vesparium (Batsch) Macbr., (H); Leocarpus fragilis (Dicks.) Rost., (H, W); Lycogala conicum Pers., (H); L. epidendrum (L.) Fr., (F); L. exiguum Morg., (H); Physarum globuliferum (Bull.) Pers., (H); P. leucopus Lk., (H); P. nutans Pers., (F, H); Trichia contorta (Ditm.) Rost., (H); T. favoginea (Batsch) Pers., (F, H); T. inconspicua Rost., (H).

PHYCOMYCETES: Synchytrium decipiens Farl. on Amphicarpa monoica (L.) Ell., (O, W).

Pyrenomycetes: Bertia moriformis (Tode) Fr. on Betula lenta L., (F); Clypeolella Leemingii (Ell. & Ev.) Theiss. on Panax aphylla L., (F, O, W); Coccomyces coronatum (Schum.) DeNot. on Quercus sp., (W); C. dentatus (Kze. & Schum.) Sacc. on Rhododendron maximum L., (O, W); Cordyceps capitata (Holmsk ex Fr.) Tul. on Elaphomyces cervinus (L. ex Gray) Schlecht., (F); C. militaris (L.) Lk., (F, O, W); Daldinia concentrica (Bolt.) Ces. & DeNot., (W); Dimerosporium Galactis Ell. & Ev. on Panax aphylla L., (F); D. Tsugae Dearn. on Tsuga canadensis (L.) Carr., (F, W); Elaphomyces cervinus (L. ex Gray) Schlecht., (F); Endothia parasitica (Murr.) And, & And, on Castanea dentata (Marsh.) Borkh., (W); Eutypa spinosa (Pers.) Nitschke on Quercus sp., (W); Gloniopsis Smilacis (Schw.) Und. & Earle on Smilax sp., (W); Glonium stellatum Muhl., (O, F); Gnomonia Coryli (Batsch ex Fr.) Awd. on Corylus rostrata Ait., (F); Hypocrea gelatinosa (Tode) Fr. on Betula sp. and Polyporus sp. on dead wood, (O, W); H. lenta (Tode) Berk. & Br., (W); H. rufa (Pers.) Fr. on Quercus sp., (W); Hypoderma commune (Fr.) Duby on Smilax sp., (W); Hypoxylon caries (Schw.) Sacc., (W); H. coccineum Bull. on Rhododendron maximum L., (W); H. cohaerens (Pers.) Fr. on Rhododendron maximum L., (W); H. marginatum (Schw.) Berk., (W); H. multiforme Fr. on Acer sp., (W); H. rubiginosum (Pers.) Fr., (O); Lasiosphaeria ovina (Pers.) Ces. & DeNot. on Betula lenta L., (F); Leptosphaeria fusispora Niessl on Cimicifuga racemosa (L.) Nutt., (W); Microsphaera Alni (Wallr.) Wint. on Quercus coccinea Muench., (W); Nectria cinnabarina (Tode) Fr., (W); N. lactea Ell. & Morg., (W); Ophiodothella Vaccini Boyd, (O); Peckiella viridis (Pers.) Sacc., (O); Podostroma alutaceum (Pers.) Atk., (F, O); Propolis faginca (Schrad.) Karst., (O); Rhytisma salicinum (Pers.) Fr. on Salix sp., (F, O, W); Rosellinia purpurcofusca (Schw.) Ell. & Ev. on Betula sp., (W); R. subiculata (Schw.) Sacc., (W); Schizothyrium Gaultheriae (Curt.) v. Hoehn, on Gaultheria procumbens L., (W); Stictis quercifolia Cke. & Ell. on Ouercus sp., (W); S. radiatus (L.) Pers., (O, W); Ustulina vulgaris Tul. on Acer rubrum L., (W); Venturia Rhododendri Tengw. on Rhododendron maximum L., (W); Xylaria corniformis Fr. on Fagus grandifolia Ehrh., (W); X. Cornu-Damae (Schw.) Berk., (F. O. W).

DISCOMYCETES: Arachnopesiza arenea (DeNot.) Sacc. on chestnut burs, (F, W); A. delicatula Fckl., (W); Calycina macrospora (Peck) Seaver, (W); Chlorosplenium chlora (Schw.) Massee on chestnut log, (F, O, S,

W); Ciboria nebulosa (Cke.) Seaver. (O); Coryne sarcoides (Jacq.) Tul. conidial stage on Fagus grandifolia Ehrh., (O, W); C. urnalis (Nyl.) Sacc., (W); Cudonia lutea (Peck) Sacc., (F, O, W); Cyathicula coronata (Bull.) DeNot., (W); Dermatea brunneo-pruinosa Zeller on Rhododendron maximum L., (W); Dermatea prunastri (Pers.) Fr., (F, O); D. purpurascens Ell. & Ev. on Castanea dentata (Marsh.) Borkh., (O, W); Geoglossum glabrum Pers., (O, W); G. hirsutum Pers., (W); ?Godroniopsis quernea (Schw.) Diehl & Cash, (W); Helotium citrinum (Hedw.) Fr., (W); H. epiphyllum (Pers.) Fr. on Acer sp., (W); H. scutula (Pers.) Karst., (W); Helvella atra Oed., (W); Helvella crispa (Scop.) Fr., (F, O); H. infula Schaeff., (O); Lachnum ciliare (Schrad.) Rehm, (W); L. virgineum (Batsch) Karst., (W); Lamprospora trachycarpa (Curr.) Seaver, (W); Leotia lubrica (Scop.) Pers., (O, W); L. lubrica f. Stevensoni (B. & Br.) Mass., (F); Microglossum rufum (Schw.) Underw., (W); Mollisia cincrea (Batsch) Karst., (W); Orbilia xanthostigma Fr., (W); Otidea grandis (Pers.) Mass., (W) or Otidea leporina (Batsch) Fckl., (F, O); Patella albida (Sch.) Seaver, (F); P. scutellata (L.) Morg., (F, O, W); Paxina hispida (Schaeff.) Seaver, (F, O, W); Pezicula acericola Peck, (O); P. minuta Peck on Viburnum alnifolium Marsh., (O, W); P. Rubi (Lib.) Niessl on Rubus odoratus L., (F, W); Phialea dolosella (Karst.) Sacc., (O, W); P. phyllophila f. fagicola Sacc. on Fagus leaves, (O, W); P. scutula var. Pteridis (Feltg.) Sacc. on ?Pteridium aquilinum L., (F); Pseudopeziza Ribis Kleb, on Ribes sp., (W): Sarcoscypha floccosa (Schw.) Sacc. on Hicoria sp., (S, W); S. occidentalis (Schw.) Sacc., (W, ?S); Spathularia velutipes Cke. & Farl., (F, O, W).

UREDINALES: Coleosporium solidaginis Thuem. on Aster acuminatus Michx., (W), on Solidago Curtisii T. & G., (W), on S. rugosa Mill., (W), Solidago sp., (O); Gymnoconia Peckiana (House) Trotter on Rubus allegheniensis Porter, (F); Gymnosporangium globosum Farl. on Crataegus sp., (F, W); Kuchneola uredinis (Lk.) Arth. on Rubus allegheniensis Porter, (F, O, W) and R. trivialis Michx., (F, W); Phragmidium Potentillae (Pers.) Karst. on Potentilla canadensis L., (F); Puccinia atropuncta P. & C. on Amianthium muscaetoxicum (Walt.) Gray, (F); P. Cypripedii Arth. on Cypripedium parviflorum Salisb., (W); P. Heucherae (Schw.) Diet. on Tiarella cordifolia L., (F, O); P. Menthae Pers. on Monarda fistulosa L., (W): P. Schedonnardi Kell. & Swingle on Muhlenbergia Schreberi J. F. Gmel., (W); P. Violae (Schum.) DC. on Viola hastata Michx., (F); Pucciniastrum Hydrangeae (B. & C.) Arth. on Hydrangea arborescens L., (F, O, W); P. Myrtilli (Schum.) Arth. on Vaccinium erythrocarpon Michx., (W); Tranzschelia Pruni spinosae (DC.) Lindr. on Prunus sp. (wild cherry), (W); Uromyces Hyperici (Spreng.) Curt., (O); Uromyces Silphii

(Burr.) Arth. on Juneus Dudleyi Wieg., (F).

Protobasidiomycetes: Calocera cornea (Batsch) Fr., (F, W); Sebacina incrustans Pers. ex Tul., (F); Tremella carneo-alba Coker on Diatrype Stigma (Hoffm.) Fr., (W); T. frondosa Fr., (O, W); Tremellodendron candidum (Schw.) Atk., (W); T. cladonia (Schw.) Burt, (W); T. pallidum (Schw.) Burt, (F, O); Tremellodon gelatinosum Scop., (F, O, W). Thelephoraceae: Aleurodiscus acerinus (Pers.) v. Hoehn. & Litsch.,

(F); A. candidus (Schw.) Burt, (O); A. Oakesii (Berk. & Curt.) Cke.,

(F, O); Corticium atrovirens Fr., (F, O); C. vagum Berk. & Curt., (O); Craterellus cantharellus (Schw.) Fr., (F, O); Cyphella capula f. flocculosa Bourd. & Galz. on Eupatorium sp., (W); Exobasidium Vaccinii Wor., (O); Hymenochaete rubiginosa (Dicks.) Lev. on Quercus sp., (W); H. spreta Peck, (O, W); H. tabacina (Sow.) Lev., (O, W); Hypochnus botryoides (Schw.) Burt, (O); H. isabellinus Fr., (O); H. ferrugineus (Pers.) Fr., (O, W); H. pannosus (Berk. & Curt.) Burt, (O); Peniophora cocineofulva (Schw.) Burt, (O); Peniophora cremea Bres., (O); P. Peckii Burt, (O); Solenia fasciculata Pers., (F); Stereum Burtianum Peck, (O); S. fasciatum Schw., (W); S. hirsutum (Willd.) Fr., (W); S. rameale (Schw.) Fr., (O, W); Thelephora vialis Schw., (F, O, W).

CLAVARIACEAE: Clavaria crocca Pers., (F, W); C. fusiformis (Sow.) Fr., (O); C. Kunzei Fr., (W); C. laciniata Schaeff., (O); C. mucida Pers., (F, O); C. ornatipes Peck, (F, O); C. pistillaris (L.) Fr., (F, O, W); C. stricta (Pers.) Fr., (O); C. subbotrytis Coker, (F); Pistillaria clavulata

Ellis, (W); Typhula filicina Peck, (F, W).

HYDNACEAE: Grandinia raduloides (Karst.) Bourd., (O); Hydnum adustulum (Bank.) Sacc. & Trott., (O); H. adustum Schw., (O); H. Caput-Ursi Fr., (O); H. coralloides Scop., (F); H. erinaceum Bull., (W); H. repandum (L.) Fr., (F, O, W); H. rufescens (Pers.) Fr., (O); H. zonatum (Batsch) Fr., (O); Odontia bicolor (A. & S.) Schw., (O); O. lactea

Karst., (O); Steccherinum ochraceum (Pers.) S. F. Gray, (W).

Polyporaceae: Cyclomyces Greenei Berk., (O, W); Daedalea confragosa (Bolt.) Fr., (W); D. quercina (L.) Fr., (W); Favolus alveolaris (DC.) Quel., (F); Fistulina hepatica (Huds.) Fr., (F, W); Fomes applanatus (Pers.) Gill., (O, W); F. connatus Fr., (F, O, W); F. fomentarius (L.) Fr., (O); F. igniarius var. laevigatus (Fr.) Overh., (O); F. pinicola (Schw.) Fr., (O); Lenzites betulina (L.) Fr., (O, W); Polyporus albellus Peck, (O); P. cinnabarinus (Jacq.) Fr., (O, W); P. frompactus Overholts, (S); P. dichrous Fr., (O); P. elegans (Bull.) Fr., (F, O); P. fragilis Fr., (O, W); P. giganteus (Pers.) Fr., (O, W); P. guttulatus Peck, (F, O); P. hirsutus (Wulf.) Fr., (O, W); P. immitis Peck, (O); P. nidulans Fr., (F); P. pargamenus Fr., (W); P. semipileatus Peck, (O); P. (Ganoderma) Tsugae (Murr.) Overh., (O); P. versicolor (L.) Fr., (O, W); Poria candidissima Schw., (O); P. crassa Karst., (F); P. semitincta (Peck) Cke., (F, O, W); P. versipora Pers., (F, S).

BOLETACEAE: Boletinus castanellus Peck, (F, O, S); B. pictus Peck, (O, S, W); Boletus affinis Peck, (F, S); B. alboater Schw., (O, S); B. americanus Peck, (O, S); Boletus auriporus Peck, (F); Boletus badius Fr., (F, S); B. bicolor Frost, (S); B. castaneus Bull. ex Fr., (O, S); B. chrysenteron Fr., (F, S); B. cyanescens Bull. ex Fr., (S); B. felleus Bull. ex Fr., (S); B. gracilis Peck, (F, S); B. granulatus (L.) Fr., (F, O, S); B. indecisus Peck, (F, S); B. miniato-olivaceus Frost, (F, O, S); B. ornatipes Peck, (S); B. pallidus Peck, (S); B. parasiticus Bull., (F); B. punctipes Peck, (O, S); B. rugosiceps Peck, (S); B. scaber Fr., (F, O, S); B. separans Peck, (S); B. subluteus Peck, (O, S); Strobilomyces strobilaceus

(Scop.) Berk., (O, S).

AGARICACEAE: Amanita bisporiger Atk., (F, O); A. Caesarea (Bull.) Fr., (O); A. chlorinosma Peck, (F, O, S); A. flavoconia Atk., (F, O, S);

A. mappa Fr., (F); A. rubescens Fr., (F, S); A. solitaria (Bull.) Fr., (O, S); A. spissa Fr., (S); A. verna Fr., (S); Amanitopsis vaginata var. fulva Sacc., (F, O, S); A. vaginata var. livida Peck, (S); Armillaria mellea (Vahl.) Fr., (O, S); Cantharellus aurantiacus Fr., (F, O, S, W); C. cinnabarinus Schw., (F, O, S); C. cibarius Fr., (O); C. infundibuliformis (Scop.) Fr., (F, O); C. tubaeformis Fr., (F); Clitocybe clavipes (Pers.) Fr., (O); C. illudens (Schw.) Sacc. (O); C. laccata (Scop.) Fr., (O); C. ochropurpurea Berk., (F, O, W); Clitopilus orcellus Fr., (O); Collybia albiflavida (Pk.) Kauff., (F); C. confluens Fr., (W); C. dryophila (Bull.) Fr., (O); C. platyphylla Fr., (F); C. radicata (Rehl.) Berk., (O); C. strictipes Peck, (O); Cortinarius armillatus Fr., (F, O); C. bolaris Fr., (O); C. corrugatus Peck, (F, O, S); C. purpurascens Fr., (F, O); C. semisanguineus Fr., (S); Crepidotus dorsalis Peck, (F, O); C. malachius Berk. & Curt. (F, O); Flammula polychroa Berk., (F, O); Galera tenera var. pilosella Peck, (O); Hygrophorus borealis Fr., (O); H. chlorophanus Fr., (O); H. conicus (Scop.) Fr., (O); H. coccineus Fr., (S); H. marginatus Peck, (S); H. miniatus Fr., (F); H. psittacinus (Schaeff.) Fr., (O); Inocybe hystrix Fr., (F); I. lilacina (Peck) Kauffm., (F, O); Lactarius camphoratus (Bull.) Fr., (O); L. cinereus Peck, (O); L. corrugis Peck, (F); L. croceus Burl., (F); L. deceptions Peck, (F, O, S); L. Gerardii Peck, (O); L. lignyotus Fr., (F); L. luteolus Peck, (F); L. Peckii Burl., (F, O); L. pergamenus (Swartz) Quél., (O); L. piperatus (Scop.) Fr., (O); L. subpurpureus Peck, (F); L. theiogalus Fr., (F, O); Lepiota ?acutisquamosa Fr., (S); L. amianthina (Scop.) Fr., (O); L. cristata Fr., (O); L. metulispora Berk. & Br. sensu Bres., (F); L. procera Fr., (S); L. rugoso-reticulata Lorin, (O, W); Marasmius androsaceus Fr., (F); M. cohaerens Fr., (F); M. confluens (Pers.) Ricken, (O); M. dichrous Berk. & Curt., (F); M. foetidus Berk. & Curt., (O); M. instititius Fr., (O, W); M. oreades Fr., (W); M. resinosus Peck, (W); M. rotula (Scop.) Fr., (O, W); M. semihirtipes Peck, (O, W); M. siccus (Schw.) Fr. (F, S, W); M. subnudus (Ell.) Peck, (O, S); Mycena Leaiana Berk., (F, O); Omphalia campanella (Bull.) Fr., (O); O. fibula (Bull.) Fr., (O); Panus stypticus (Bull.) Fr., (O, W); Paxillus involutus Fr., (F); Paxillus rhodoxanthus Schw., (F); Pholiota acericola Peck, (O); P. Johnsoniana Peck, (O); P. squarrosoides Peck, (O); P. subsquarrosa Fr., (F); Pleurotus applicatus Fr., (F, W); P. ostreatus Fr., (W); P. porrigens (Pers.) Fr., (O); P. sapidus Fr. (F, S); Pluteus cervinus (Schaeff.) Fr., (O, S); Psalliota diminutiva Peck, (F, O); Psilocybe foenisecii (Pers.) Fr., (O); Russula crustosa Peck, (F, O, S, W); R. decolorans Fr., O); R. emetica Fr., (S); R. flava Romell, (O, S); R. foetens Fr., (F, O, S); R. fragilis Fr., (F, S); R. lactea Pers., (O); R. sericeonitens Kauffm., (O); R. simillima Peck, (O); R. variata Bann. & Peck, (O); R. ?violaceus Quél., (F); Schizophyllum commune Fr., (S, W); Tricholoma sulphureum Fr., (S); Trogia crispa Fr., (W).

GASTEROMYCETES: Bovista pila Berk. & Curt., (W); Calostoma cinnabarina Desv., (F, O, W); C. Ravenelii (Berk.) Mass., (F); Calvatia cyathiformis (Bosc.) Morg., (W); Crucibulum vulgare Tul., (W); Cyathus stercoreus (Schw.) DeToni (W); C. striatus Willd., (W); Lycoperdon atropurpureum Vitt., (W); L. Curtisii Berk., (F); L. fuscum Bon., (F); L. gemmatum Batsch, (O, W); L. marginatum Vitt., (F, O); L. pyriforme Schaeff., (F); L. subincarnatum Peck, (W); Scleroderma aurantia (Vaill.)

Pers., (O, W); Sphaerobolus stellatus Tode, (S, W).

FUNGI IMPERFECTI: Anthina pallida DeBy on Rhododendron maximum L., (W); Cephalosporium Acremonium Cda. on Lycogala epidendrum, (F); Cercospora ageratoides Atk., (O); C. clavata (Gerard) Cke., on Asclepias sp., (F); C. smilacina Sacc., (O); C. Violae Sacc., (W); Cercosporella cana (Pass.) Sacc., on Erigeron canadensis L., (W); Cladosporium sp. on Rubus odoratus L., (W); Cryptostictis Mariae (Pk.) Sacc. on Rhododendron maximum L., (W); Cylindrosporium acerinum (Peck) Dearn. & House, (O); C. saccharinum Ell. & Ev., (W); Darluca Filum (Bivon.) Cast. on Phragmidium Potentillae (Pers.) Karst., (F), on Puccinia Schedonnardi Kell. & Swingle, (W), on P. Menthae Pers., (W), on Uromyces Silphii (Burr.) Arth., (F), host not stated, (O); Dendrodochium compressum Ell. & Ev., (F, O, W); Discosia rugulosa B. & C. on Carya sp., (W); Entomosporium maculatum Lev. on Amelanchier sp., (W); Fusicladium Robiniae Shear on Robinia Pseudo-Acacia L., (W): Gelatinosporium betulinum Peck on Betula lenta L., (O, W); Gonatobotryum maculicola (Wint.) Sacc. on Hamamelis virginiana L., (F, O, W); Marssonia Martinii Sacc. & Ell. on Quercus sp., (W); M. ochroleuca Berk. & Curt. on Castanea dentata (Marsh.) Borkh., (W); Myocopron Smilacis (DeNot.) Sacc. on Smilax sp., (W); Pestalotia macrotricha Kleb. on Rhododendron maximum L., (W); Pestalotia sp. on Osmunda sp., (W); Phoma castanea Peck, (O); Phyllosticta minutissima Ell. & Ev. on Acer sp., (F); P. vagans Peck on Convalaria majalis L., (W); Polythrincium trifolii (Kze.) (O, W); Ramularia Oxalidis Farl. on Oxalis Acctosella L., (W); Septoria Rubi West., (W); S. Rubi var. pallida Ell. & Holway, (O); Sphaeronema acerina Peck, (O); Sporocybe Azaleae (Pk.) Sacc. on Rhododendron maximum L., (W); Sporodesmium concinnum Berk., (F, O); S. peziza Cke. & Ell. on Castanea dentata (Marsh.) Borkh., (W); S. polymorphum Cda., (F); Stemphylium sp. on Rhododendron maximum L., (W); Stephanoma strigosum Wallr. on Lachnea hemispherica, (W); Stilbum giganteum Peck on Acer, (F, W); Streptothrix atra Berk. & Curt. on Fagus grandifolia L., (W); Thyrsidium botryosporium Mont. on Fagus grandifolia L., (F).

In addition to the foregoing species, Dr. Walter H. Snell collected the following in Blacksburg, Virginia: Clitocybe ochrosperma Berk., (S); Psalliota sylvatica Fr., (S); Boletus Betula Schw., (S); B. erythropus Fr.,

(S).—DAVID H. LINDER.



